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NITRATE REMOVAL FROM WATER AT THE WATER-MUD INTERFACE IN WETLANDS

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The oxidized and reduced layers in flooded soil were characterized by vertical distribution of the oxidation-reduction (redox) potential and concentrations of manganous manganese, ferrous iron, sulfide, nitrate and ammonium. The apparent thickness of the oxidized layer was different when evaluated by the distribution of these various components in the profile. Flood water NO_3 removal rates of intermittently-flooded freshwater swamp soils and continuously-flooded saline marsh soils indicated that the area of NO_3 reduction was in the soil, added organic matter increased the rate of NO_3 reduction and the reduction rate was approximately twice as fast in the marsh soil as in the swamp soil. Atmospheric O_2 over a flooded soil increased denitrification up to a concentration of approximately 20%. The N loss appeared to be related to the thickness of the sediments aerobic layer. Ammonium nitrogen in a flooded soil exposed to O_2 from the water column undergoes sequential nitrification and denitrification. Ammonium nitrogen is nitrified in the aerobic zone, diffuses to the anaerobic zone where it is denitrified to N_2 and N_2O and then diffuses from the system. Oxygen reduction rates, NO_3 reduction rates and the effects of added O_2 on NO_3 reduction and redox potential in flooded soils were investigated and indicated that NO_3 added to floodwater rapidly disappeared, O_2 loss from sediments occurred rapidly and NO_3 reduction was not inhibited by up to 16 ppm O_2 dissolved in soil suspensions.

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a. DESCRIPTORS		b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Nitrate*	Swamp		02D
Ammonium*	Microbial		06ACF
Oxidation-Reduction*	Diffusion		07B
Nitrification*	Organic Matter		08H
Denitrification*	Oxygen		
Soils*			
Marsh			

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ABSTRACT

I. The oxidized and reduced layers in flooded soil were characterized by vertical distribution of the oxidation-reduction (redox) potential and concentrations of manganous manganese, ferrous iron, sulfide, nitrate, and ammonium. Redox potential was measured with a special motor-driven assembly which advanced a platinum electrode at a rate of 2 mm/hour through the flooded soil profile. Vertical distribution of reduced forms of manganese, iron, and sulfur and of nitrate and ammonium was determined by freezing and slicing the flooded soil into segments 1 or 2 mm thick. The apparent thickness of the oxidized layer was different when evaluated by the distribution of the various components in the profile, with the sulfide profile indicating the thickest oxidized zone, the manganese profile indicating the thinnest oxidized zone, and the iron profile showing an intermediate thickness. The thickness of the oxidized layer increased with duration of flooding.

II. The floodwater NO_3^- removal rate of intermittently-flooded fresh water swamp soils and continuously-flooded saline marsh soils of southern Louisiana was quantitatively characterized in a laboratory study. Of the two areas studied, the marsh area was the more effective sink for NO_3^- contaminated waters with an average initial removal rate of 9.15 ppm N/day. After correcting for the rate of NO_3^- diffusion, the microbial NO_3^- removal rate was calculated to be 7.64 ppm N/day. The swamp soil had a removal rate of 4.38 ppm N/day. The microbial NO_3^- removal rate for this area, after correcting for diffusion, was 2.50 ppm N/day. Studies on samples of floodwater separated from the soil showed the active site of microbial NO_3^- reduction to be the soil-water interface or within the soil, but not in the floodwater. Additions of organic matter to a mineral soil flooded for rice (Oryza sativa L.) culture decreased the thickness of the aerobic-anaerobic zone at the soil-water interface and increased the rate of NO_3^- reduction.

III. The concentration of atmospheric O_2 over a flooded soil is a factor in determining the amount of N lost by denitrification. Large increases in N loss occurred from the first few increments of oxygen with little further loss occurring above 20 percent O_2. The thickness of the aerobic layer was also governed by the amount of O_2 in the air. Nitrogen loss was generally related to the thickness of the aerobic layer, even though appreciable loss occurred at 5 and 10 percent O_2 where the aerobic layer was relatively thin.

IV. Ammonium nitrogen in a flooded soil or sediment exposed to oxygen from the water column undergoes sequential nitrification and denitrification. Ammonium in the aerobic surface layer of soil or sediment is

nitrified and the resulting concentration gradient between this layer and the underlying anaerobic layer causes ammonium to diffuse upward into the aerobic layer where it also undergoes nitrification. Nitrate produced in the aerobic layer then diffuses down into the anaerobic layer where it is denitrified to N_2 and N_2O. Approximately one-half of the nitrogen involved in the nitrification-denitrification process is ammonium originally present in the surface aerobic soil or sediment layer with the remainder diffusing up from the underlying anaerobic layer. If oxygen is absent or limiting, nitrification will either not occur or will occur at a lower rate, resulting in a reduced amount of nitrate available for the denitrification process.

V. The O_2 reduction rates, NO_3^- reduction rates, and the effects of added O_2 on NO_3^- reduction and redox potential in four flooded or intermittently flooded soils from the swamp and coastal marshes of Louisiana were quantitatively characterized in a laboratory study. The NO_3^- added either to the shallow floodwater or mixed with the soil in a suspension rapidly disappeared. Eighty to ninety ppm NO_3^- was reduced and disappeared from the soil suspensions in 1 to 4 days and from the floodwater over a soil in 10 to 20 days. No NO_3^- was lost from floodwater removed from the soils. Oxygen depletion in the soil suspensions occurred in 15 minutes to 4 hours. Redox potential curves exhibited a characteristic inflection after O_2 disappearance in all soils studied. Nitrate reduction did not appear to be inhibited by as much as 16 ppm O_2 dissolved in the soil suspensions.

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SECTION I

INTRODUCTION

Flooding causes marked changes in some of the chemical and biological processes taking place in the soil. These changes are caused by the curtailment of atmospheric oxygen entering the soil. In drained soils there are enough air-filled pores to permit adequate diffusion of atmospheric oxygen into the soil. When these soil pores are filled with water and the soil surface is covered with several inches of water, however, the amount of oxygen reaching the soil surface is reduced by a factor of over 10,000. Even though the supply of oxygen reaching the soil is greatly curtailed by flooding, microbial respiration is not diminished. The imbalance between the oxygen requirement of the soil and the oxygen supply reaching the soil surface results in a rapid depletion of soil oxygen within hours after flooding. The only part of a flooded soil that is oxygenated is a thin layer at the soil surface since oxygen diffusing through the flood water penetrates only a short distance into the soil before it is consumed (see Figure 1). An important property of a flooded soil is the thickness of this surface oxygenated layer. In soils that have a low microbial requirement for oxygen this layer is relatively thick, 2 to 5 centimeters, while in soils that have high oxygen requirement the layer may be no thicker than 1 to 2 millimeters. The oxygen requirement is usually governed by the supply of decomposable organic matter.

In the surface oxygenated or aerobic soil layer, microbial and chemical conditions are very much like those in the drained soil. In the underlying oxygen-free soil layer pronounced biological and chemical changes are set

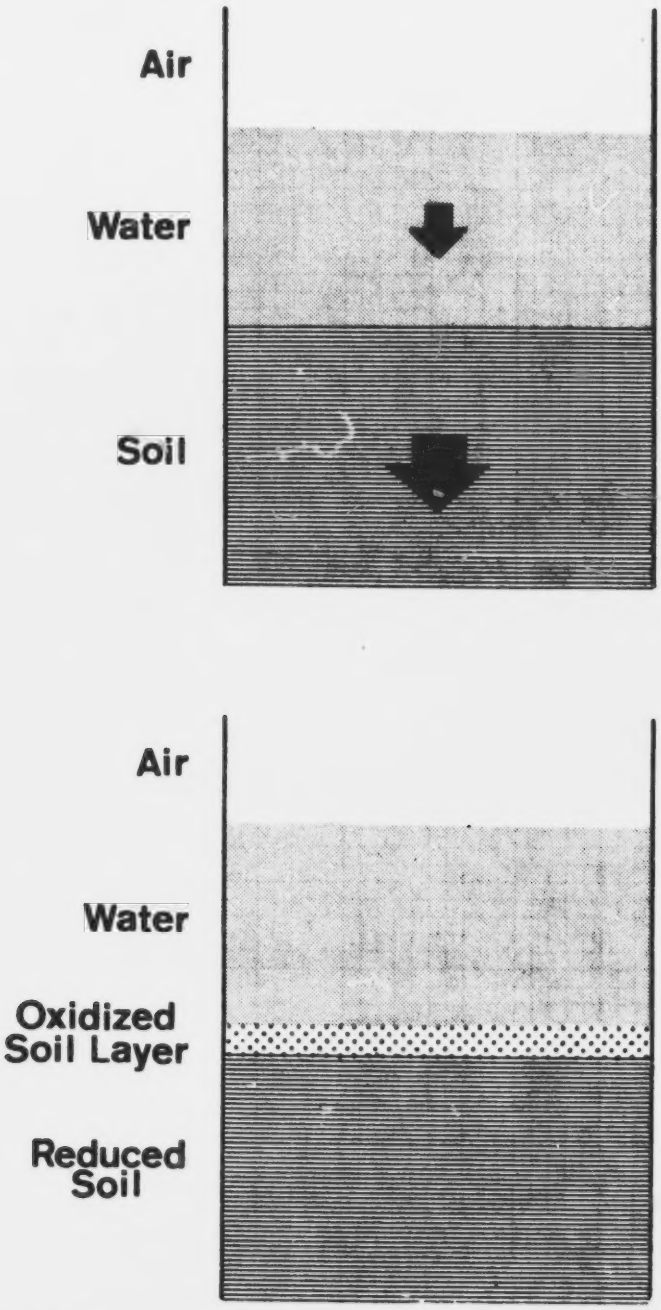


Figure 1. (Top) A diagram showing the relative rate of oxygen movement through the flood water (small arrow) and potential consumption rate of oxygen in the soil (large arrow). Reduced conditions develop in the soil when the oxygen supply through the flood water is not sufficient to meet the requirements for oxygen in the flooded soil.
(Bottom) Differentiation of a waterlogged soil into a surface oxidized or aerobic layer and an underlying reduced or anaerobic layer as a result of a limited oxygen supply reaching the soil surface.

in motion when oxygen disappears. As long as oxygen is present oxidized components of the soil such as nitrate, manganic compounds and ferric compounds are not biologically or chemically reduced. After oxygen disappears following flooding, many soil microorganisms are able to substitute one or more of these oxidized chemical components for the oxygen required in respiration. Nitrate, the higher oxides of manganese, and hydrated ferric oxide are reduced if oxygen becomes absent or limiting and if an energy source (organic matter) is available to the microorganisms. Nitrate and manganic compounds are readily reduced since the energy required for their reduction is low and a number of species of microorganisms are capable of carrying out this process. Ferric compounds are more difficult to reduce but the large amount of reducible ferric iron in most soils makes ferric compounds an important oxidation-reduction component of the soil. These reactions are carried out by facultative anaerobes, microorganisms which can substitute other reducible compounds for respiratory oxygen. Sulfate can also be reduced to sulfide by anaerobic microorganisms but this reaction is carried out only under strictly anaerobic conditions by a few species of microorganisms.

The difference in ease of reduction of the inorganic oxidation-reduction systems in the soil results in a more or less sequential reduction of the various components following flooding. Free oxygen in the soil is reduced first and is at least partially depleted before nitrate and manganese compounds begin to be reduced. Ferric compounds are reduced after nitrate and manganic manganese and, if no oxygen enters the soil, sulfate will be reduced to sulfide. Almost always, however, all oxygen and nitrate have disappeared from the soil before iron reduction begins. The availability of

an energy source and the absence of oxygen are the only necessary requirements for these reduction reactions to occur, outside of favorable environmental conditions, since the microorganisms that carry out these reactions occur in almost all soils, even those where flooding has not been known to take place.

Nitrogen reactions in flooded soils differ markedly from those of well drained soils. In well drained soils decomposition of organic matter releases ammonium nitrogen which is then oxidized biologically to nitrate. In flooded soils ammonium nitrogen accumulates since the conversion of ammonium to nitrate cannot take place in the absence of oxygen.

Not only is nitrate not produced in any part of a flooded soil except the thin surface aerobic layer, nitrate added to a flooded soil is subject to denitrification which results in loss of nitrogen from the soil as N_2 or N_2O.

Denitrification losses of nitrogen are undesirable in lowland rice agriculture since nitrogen that might be taken up by the crop is lost from the soil, however such losses from the soil-water system may be beneficial in removing toxic levels of nitrate from water or soil. This process is also extremely important in maintaining the nitrogen balance of the earth, since denitrification in lakes, oceans, swamps, marshes, and both flooded and nonflooded soils constitutes the major mechanism by which elemental nitrogen is returned to the atmosphere.

The objectives of the research carried out in this project were: (1) to find out how rapidly, how completely, and by what mechanism nitrate is removed by biological reduction from shallow surface water in swamps, marshes and flooded soils, and (2) to determine the oxidation-reduction properties

of the water-mud interface that control or influence the reduction of nitrate to nitrogen gas.

SECTION II

CHARACTERIZATION OF THE OXIDIZED AND REDUCED ZONES IN FLOODED SOIL

The presence of a surface oxidized layer and an underlying reduced layer at the surface of a flooded soil has important effects on biological and chemical transformations in the soil. Microbiological processes taking place in the oxidized zone are similar to those carried out in drained, aerated soil by aerobic microorganisms while the processes taking place in the underlying oxygen-free reduced layer involve facultative and true anaerobes. Most of the inorganic redox systems in soils occur in the oxidized form in the surface oxygenated zone (ferric iron, manganic manganese, nitrate nitrogen, sulfate sulfur) and in the reduced form in the underlying oxygen-free zone (ferrous iron, manganous manganese, ammonium nitrogen, sulfide sulfur). For the purposes of this study the oxidized layer was considered to be the surface layer of soil containing little or no reduced iron, manganese, and sulfur and the reduced layer was considered to be the layer under the oxidized layer in which ferrous iron, manganous manganese, and sulfide exist. Inorganic nitrogen is present as nitrate in the oxidized layer and as ammonium in the reduced layer (Pearsall and Mortimer, 1939).

The differentiation of a flooded soil or sediment into two distinct zones as a result of limited oxygen penetration into the soil material was first described by Pearsall and Mortimer (1939) and first investigated thoroughly by Mortimer (1941, 1942). The supply of oxygen at the soil surface and the consumption rate of oxygen in the soil were recognized as the

major factors determining the thickness of the oxidized surface layer.

The two layers existing at the surface of flooded soil can also be characterized by differences in the oxidation-reduction or redox potential. The various inorganic and organic redox systems in the soil contribute to this potential. Mortimer (1941, 1942) used a special assembly of platinum electrodes to obtain a profile of redox potential in lake muds. He found that high oxygen consumption in the mud was associated with a thin oxidized layer and that low oxygen consumption was associated with a thick oxidized layer. Gee (1950) and Alberda (1953) demonstrated the presence of a thin oxidized layer underlain by a reduced layer in a flooded soil profile.

Although the oxidized and reduced layers can be characterized by redox potential profiles, the distribution of oxidized and reduced chemical components in these layers would provide better information on the exact nature of the processes going on in the two layers of a flooded soil. Pearsall and Mortimer (1939) measured various redox components in the water above a lake bottom, in the oxidized surface mud layer, and in the underlying oxygen-free mud and found that the surface layer of mud contained the oxidized forms of iron, inorganic nitrogen and sulfur while the underlying mud contained reduced forms of these elements. Howeler and Bouldin (1971) measured the distribution of various forms of iron in a flooded profile and showed that ferric iron was present in the oxidized layer and absent in the reduced layer while ferrous iron was absent in the oxidized layer and present in the reduced layer.

The study reported here was designed (1) to develop a method for measuring the oxidation-reduction or redox potential vertically through

the soil profile in order to determine the thickness of the oxidized layer and (2) to determine the distribution of the reduced form of reducible soil components such as inorganic manganese, iron, sulfur, and the distribution of nitrate and ammonium nitrogen within the oxidized and reduced layers.

MEASUREMENT OF REDOX POTENTIAL PROFILES IN FLOODED SOIL

A technique was developed by which a small platinum electrode (approximately 1 mm long) was driven downward through an undisturbed flooded soil at a rate of 2 mm/hour. The electrode was constructed by sealing 18 gauge platinum wire into 5 mm diameter pyrex tubing with 1 mm of the wire to complete the cell. The platinum and calomel electrodes were connected to a vacuum tube voltmeter (Beckman Zeromatic pH meter) and the output from the meter recorded on a Sargent MR recorder with a chart speed of 10.16 cm/hour (4 inches/hour). With this arrangement 2.54 cm (1 in) of chart was equivalent to 0.5 cm depth. A sketch of the system is shown in Figure 2. The platinum electrode was positioned 5 ± 0.5 mm above the surface of the soil at the beginning of each profile measurement.

By use of this system it was found in a laboratory experiment that differentiation in the redox potential of a Crowley silt loam surface soil developed rapidly after submergence, Figure 3. The redox profile measured after only one day of flooding showed little change from the soil surface downward because reducing conditions had not had time to set in, but a week of submergence caused a marked change with depth. If it is assumed that redox potential values greater than + 200 mV (at pH 7) denote oxidized conditions and values less than this value denote reduced conditions

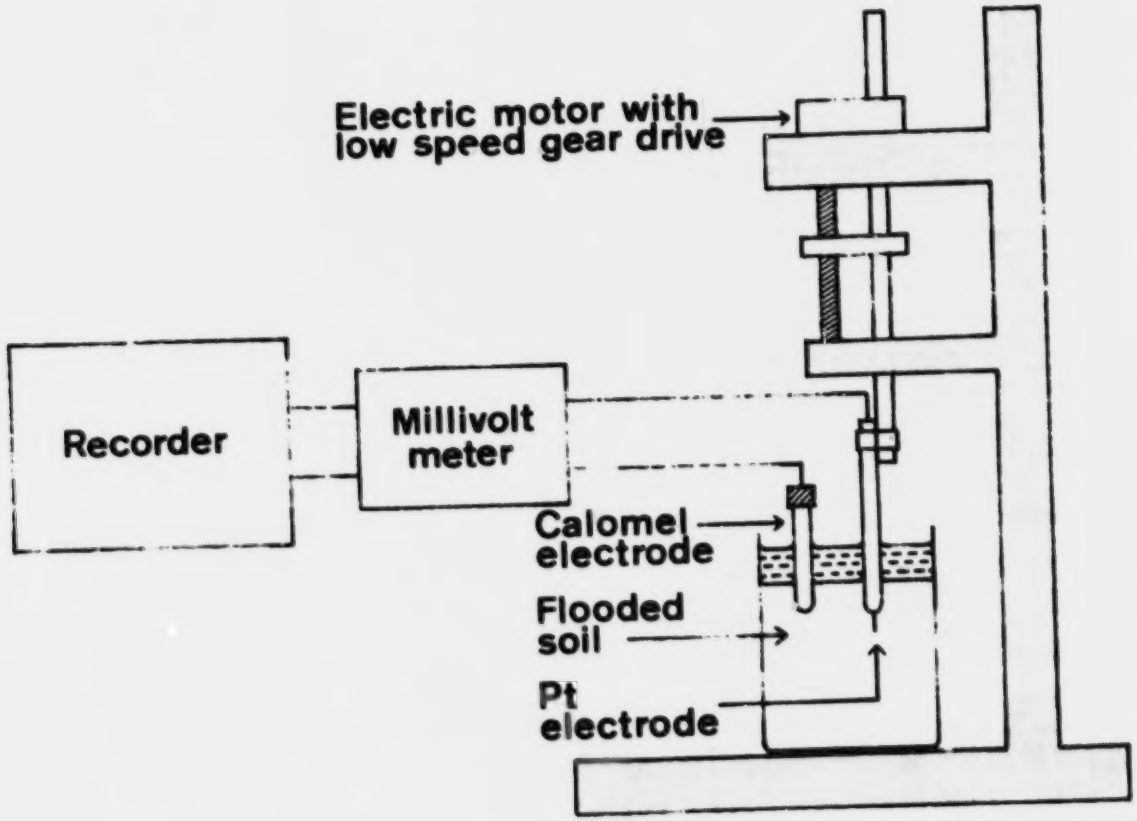


Figure 2. Apparatus used for measuring redox potential profiles in flooded soils.

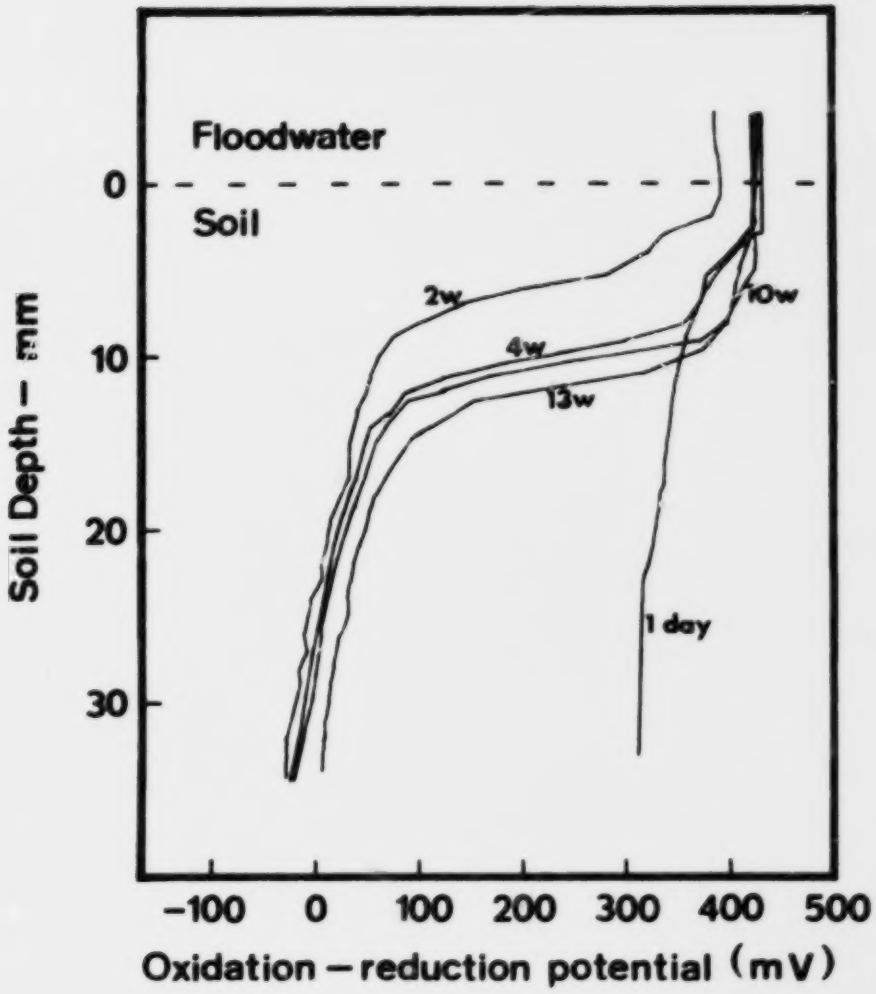


Figure 3. Oxidation-reduction (redox) potential profiles in flooded Crowley silt loam at various times after flooding. Incubation times shown are 1 day to 13 weeks.

(Pearsall, 1938; Patrick and Mahapatra, 1968), it can be readily seen that the depth of the surface oxidized layer increased with time of incubation. For the sample shown the thickness of the oxidized layer increased from approximately 6 mm at 2 weeks to about 12 mm after 13 weeks incubation. This increase with time was apparently due to the decline in the rate of organic matter decomposition which permitted oxygen from the overlying flood water to penetrate deeper into the soil before being reduced. All depths of the flooded soil had a pH value of approximately 6.8.

The method developed appears to provide a reliable way to characterize the oxidized and reduced layers of flooded soil in terms of the thickness of the oxidized layer and the relative redox potential values in both layers. The sharpness of the boundary can also be ascertained from the redox potential curve. Most of the change in redox potential occurred within a 8 mm zone. Even though the slow rate of advancement of the electrode should have provided a reasonable time for the electrode to reach near-equilibrium conditions at any given depth, the slow negative drift of a platinum electrode when placed in a biological reducing medium caused the low redox potential values (those below about + 100 mV) to be slightly higher than they would have been if the electrode had been left at a given depth for several hours.

After obtaining redox potential-depth curves such as those shown in Figure 3, the dividing line between the aerobic surface layer and the underlying anaerobic layer was estimated. The midpoint between the two points of maximum change in slope was chosen. It is interesting to note that this point occurred in all cases at approximately + 200 mV, a redox potential

(at pH 7) usually considered to be in the transition zone between aerobic and anaerobic soil conditions.

DISTRIBUTION OF INORGANIC REDUCIBLE SUBSTANCES IN FLOODED SOILS

The oxidized-reduced double layer was characterized by measuring the vertical distribution of the reduced form of several inorganic components, manganous manganese, ferrous iron, and sulfide. Reduced forms of manganese and iron are relatively soluble and can be rather easily extracted from the soil, while the oxidized forms of these elements are insoluble and difficult to extract. Sulfide can also be readily extracted and analyzed.

Samples of Crowley silt loam surface soil were placed in 113.4 g (4 oz)--cylindrical polyethylene containers (which were also used for the redox potential measurements) and submerged under approximately 2.54 cm (1 in) of water. For the sulfide experiment ^{35}S-labeled sulfate was uniformly mixed with the soil before flooding. No additional sources of iron and manganese were added. Samples were incubated for various periods ranging from 1 day to 13 weeks, rapidly frozen, and then sectioned with a large microtome into horizontal slices 1 or 2 mm thick. Freezing did not disrupt the soil as much in the plastic containers as in glass containers. The soil samples were immediately extracted for the ions to be measured. Manganese and Fe^{2+} were extracted with normal sodium acetate of pH 2.8, followed by appropriate chemical tests for the two elements (manganese by atomic absorption spectrometry and iron by the colorimetric dipyridyl method). Separate samples were incubated for the sulfide study. All of the sulfide in the thin sections was converted to hydrogen sulfide

and collected in dilute sodium hydroxide solution following acidification of the soil with sulfuric acid by use of a modified Conway diffusion cell. Radioactive sulfide was determined by a flowing gas proportional G. M. Counter. It was found convenient to use this tracer technique to follow sulfide-distribution because of the difficulty of analyzing the small amount of total sulfide in the approximately 1 g soil sections.

Manganous manganese distribution in a flooded soil profile is shown in Figure 4. As early as 1 day after submergence there was a slight increase in reduced manganese below the 5-cm depth. The amount of reduced manganese reached a maximum after 1 week. As the incubation period increased the thickness of the oxidized layer (or the layer in which manganous ion was virtually absent) also increased. For long periods of waterlogging it was apparent that oxygen was penetrating deeper into the soil and increasing the thickness of the manganous-free zone.

The profiles of ferrous iron (Figure 5) differed from the manganese profiles in two important respects. First, the thickness of the oxidized layer relatively free of the reduced form of iron was much greater than for manganese, and second, no iron reduction was evident 1 day after flooding and very little reduction had occurred after one week. Maximum reduction was reached 8 weeks after flooding. As with the manganese profiles, the longer the period of incubation, the deeper the oxidized or ferrous-free layer.

Sulfide distribution following flooding is shown in Figure 6. Sulfate reduction, similar to iron reduction, was relatively slow to develop, with maximum sulfide in the anaerobic layer occurring after 8 weeks of incubation. The thickness of the oxidized (sulfide-free) layer was greater than for iron.

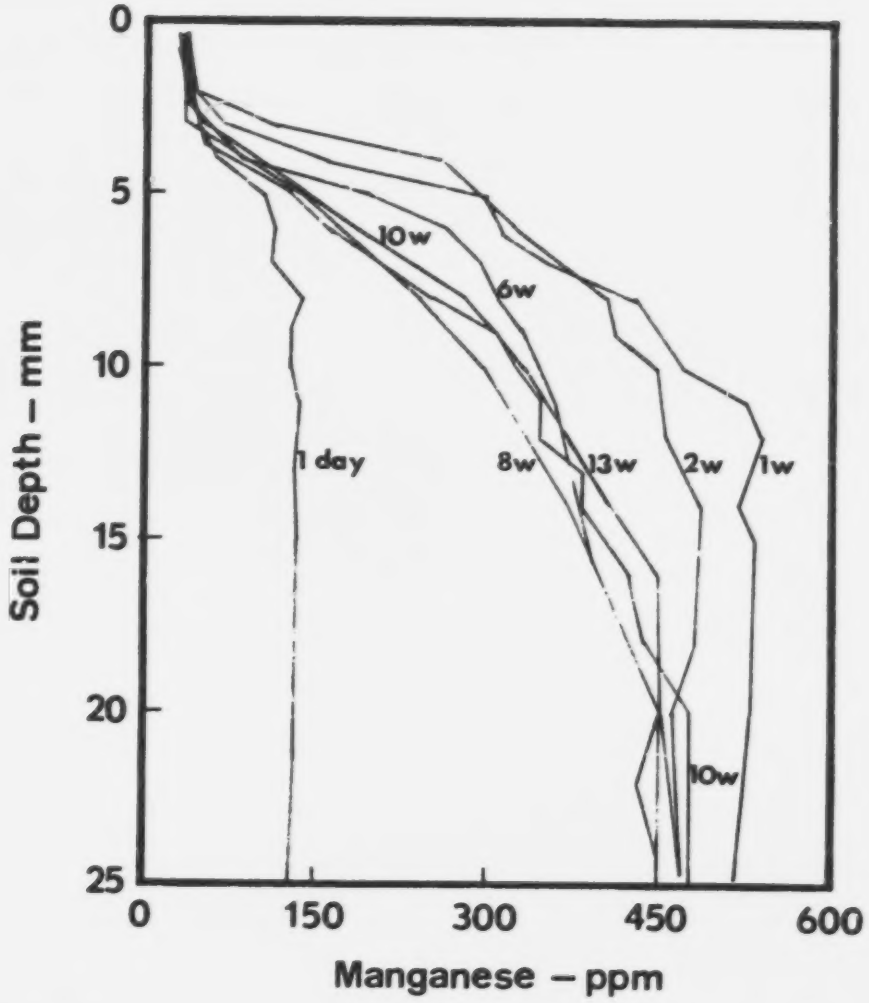


Figure 4. Vertical distribution of reduced manganese (Mn^{2+}) in a flooded Crowley silt loam at various times after flooding. Incubation times shown are 1 day to 13 weeks.

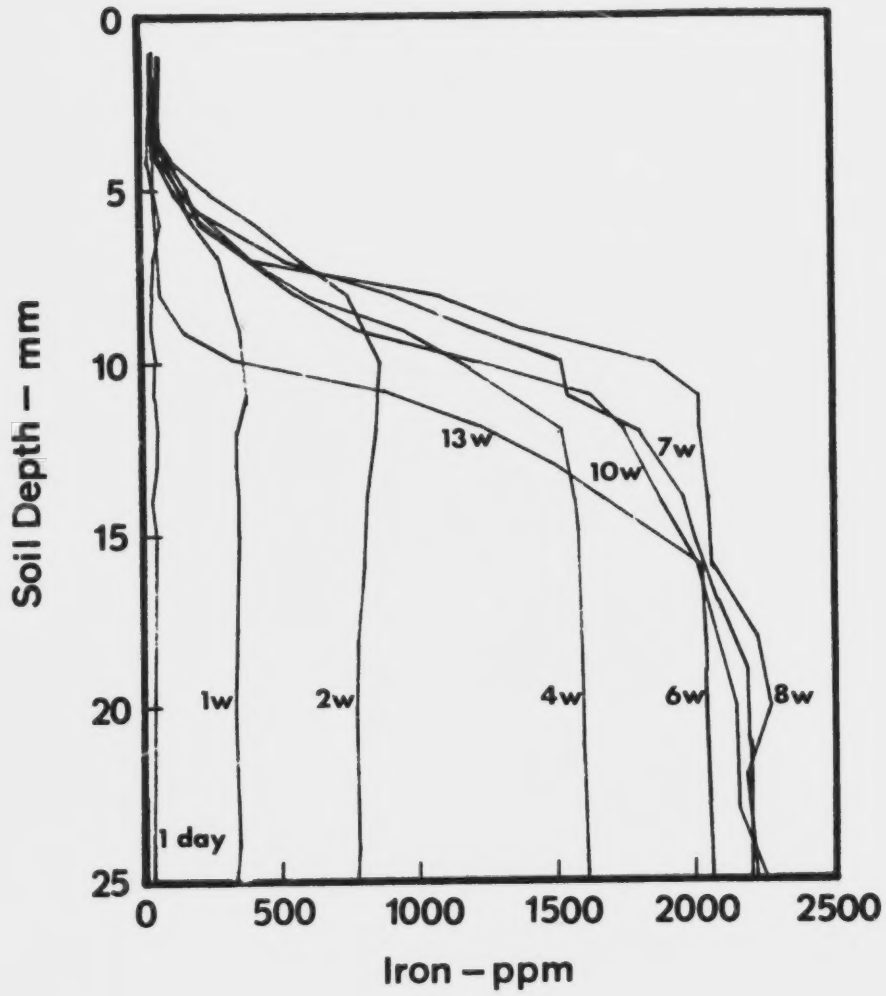


Figure 5. Vertical distribution of reduced iron (Fe^{2+}) in a flooded Crowley silt loam at various times after flooding. Incubation times shown are 1 day to 13 weeks.

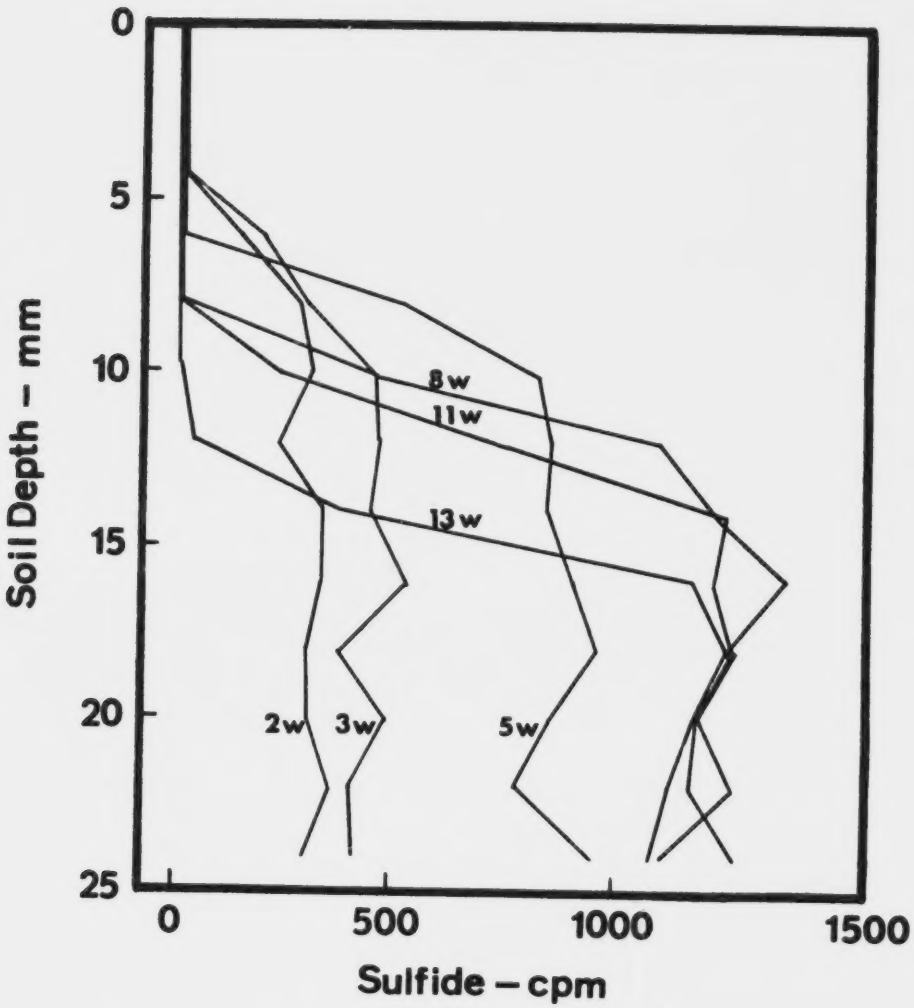


Figure 6. Vertical distribution of reduced sulfide (S^{2-}) in a flooded Crowley silt loam at various times after flooding. Incubation times shown are 2 weeks to 13 weeks.

The surface oxidized layer probably serves as an effective sink for the reduced forms of manganese, iron, and sulfur since manganous, ferrous, and sulfide ions diffusing upward to this zone would be rapidly oxidized to insoluble manganic and ferric compounds and to elemental sulfur.

DISTRIBUTION OF NITRATE AND AMMONIUM NITROGEN IN FLOODED SOIL

Nitrate and ammonium distribution in the flooded profile was determined using an experimental procedure similar to that used for manganese, iron, and sulfide. Samples of the same Crowley silt loam, into which 200 ppm N as ammonium sulfate was well mixed, were incubated under flooded conditions in polyethylene containers, frozen, and sectioned as described above. A water extract of the 2-mm sections was analyzed for nitrate by the phenoldisulphonic acid method. Ammonium in the soil was extracted in a modified Conway cell with dilute sodium hydroxide and the ammonia absorbed in dilute acid and determined by nesslerization.

The distribution of ammonium and nitrate nitrogen in the flooded soil profile is shown in Figures 7 and 8. Ammonium nitrogen became depleted in the surface layer, decreasing from 200 ppm to 50 ppm in 3 weeks in the surface 8 mm. There was a gradual increase in ammonium nitrogen with depth to about 15- to 20-mm depth. Apparently ammonium nitrogen was depleted from the upper part of the reduced soil zone by diffusion to the oxidized layer where nitrification could occur.

Although nitrate accumulated in the surface oxidized layer the maximum concentration was less than 10 ppm. There appeared to be a deepening of the nitrate-containing layer with time down to the 15- to 20-mm depth. The reason for the low concentration of nitrate in the oxidized layer was very

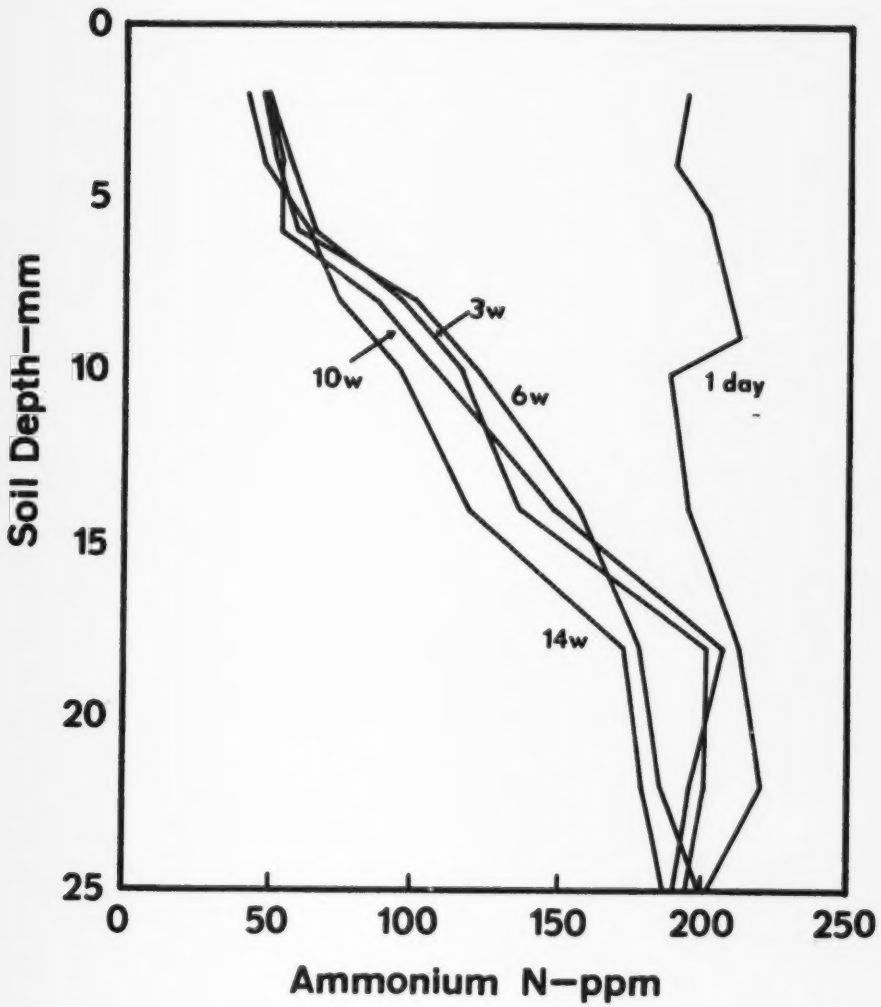


Figure 7. Vertical distribution of ammonium in a flooded Crowley silt loam at various times after flooding. Incubation times shown are 1 day to 14 weeks.

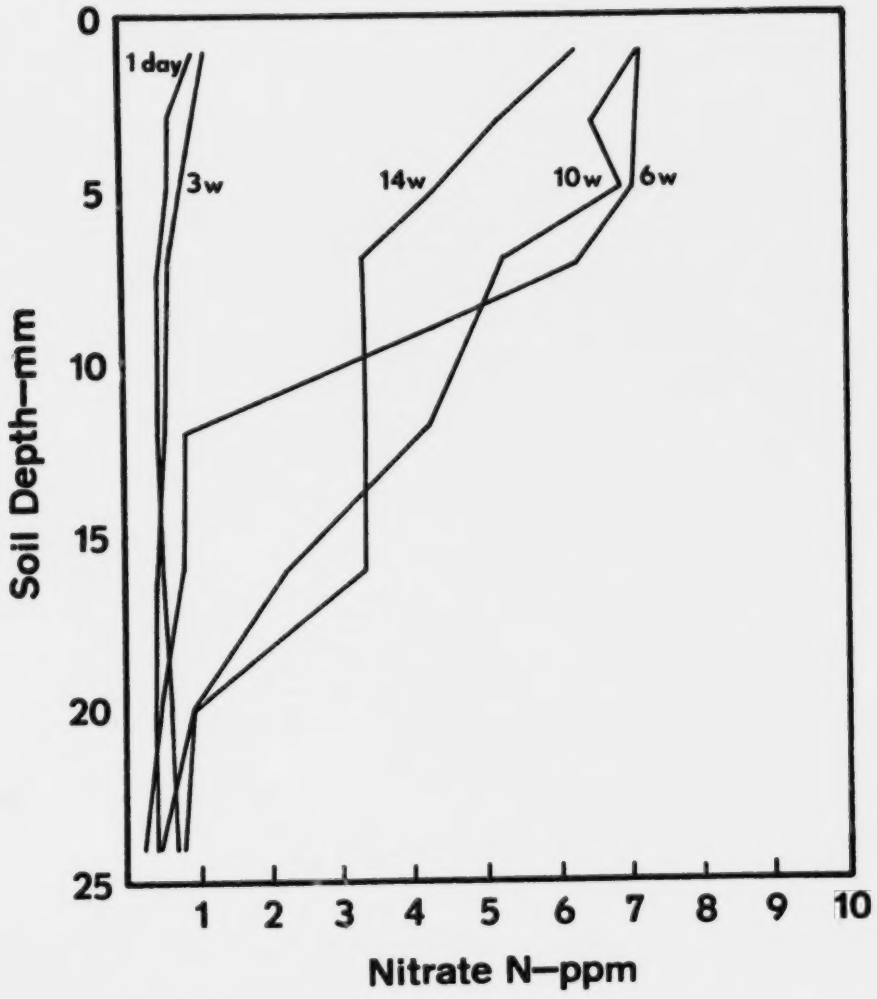


Figure 8. Vertical distribution of nitrate in a flooded Crowley silt loam at various times after flooding. Incubation times shown are 1 day to 14 weeks.

likely due to nitrate being lost through diffusion and denitrification at the same time that it was being produced from ammonium oxidation. Because of its mobility nitrate formed in the oxidized layer readily diffuses down into the anaerobic layer where denitrification will occur. The process: ammonium diffusion from the reduced layer to the oxidized layer → ammonium oxidation to nitrate (nitrification) → nitrate diffusion from the oxidized layer to the reduced layer → denitrification, can explain the decrease in ammonium in the oxidized layer and the small amount of nitrate present in the aerobic layer at any one time. Such a process will also explain the large loss of nitrogen from flooded soils which receive additions of ammonium nitrogen (Tusneem and Patrick 1971; Broadbent and Tusneem 1971).

CONCLUSIONS

The thickness of the oxidized surface layer of a flooded Crowley silt loam soil was least for manganese, intermediate for iron, and greatest for sulfide. The differences in thickness of the oxidized layer could be predicted on the basis of the ease of reduction of the compounds of these elements since manganese was easiest, iron intermediate, and sulfate most difficult to reduce. Sulfate required a lower redox potential before reduction could occur and hence was reduced at a lower depth in the flooded soil. This progressive increase in thickness of the surface oxidized layer can be best seen in Figure 9. It should be noted that the redox potential profile corresponded most closely to the iron profile in terms of the thickness of the oxidized layer.

The oxidized layer was depleted of ammonium nitrogen and accumulated

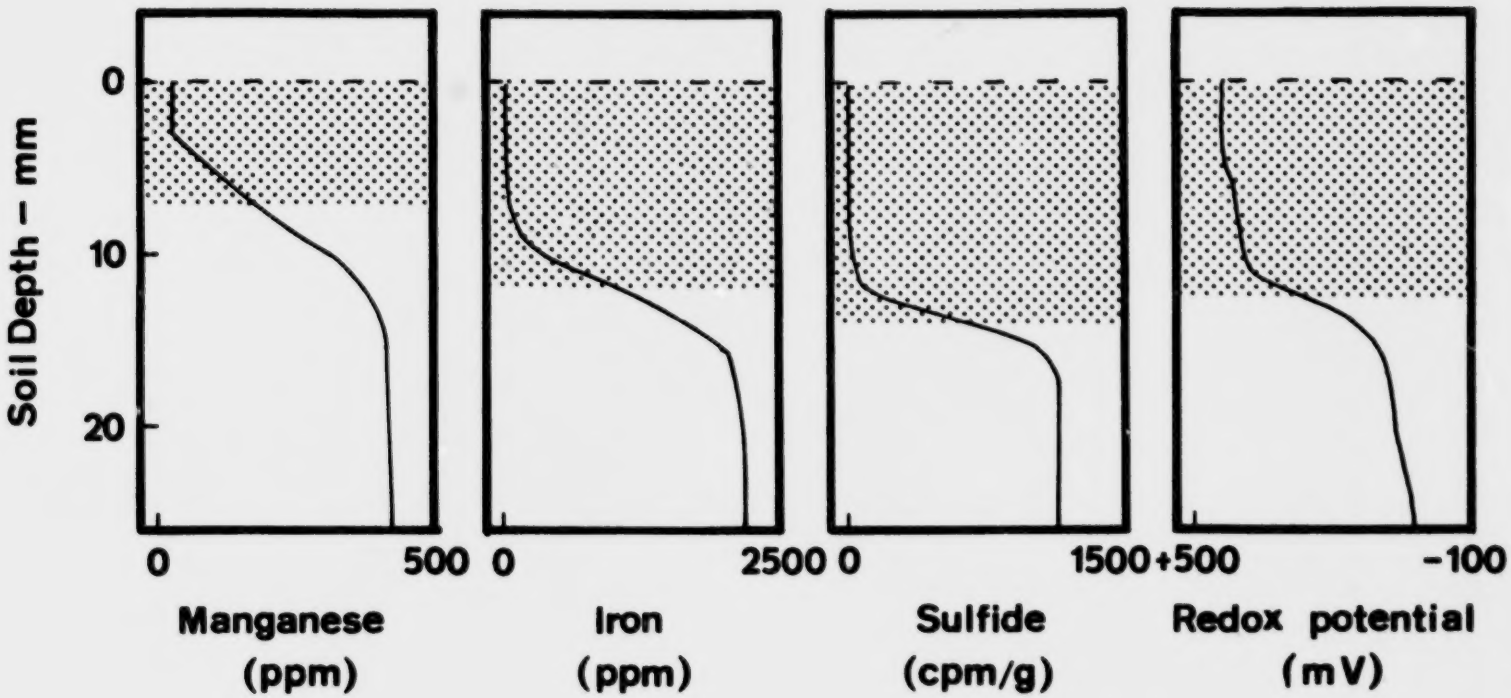


Figure 9. Thickness of the surface oxidized layer as measured by the manganese, iron, sulfur, and redox potential profiles after 13 weeks flooding.

nitrate, although the amount of nitrate did not account for the large decrease in ammonium. Nitrification and denitrification were probably both proceeding at the same time in the oxidized and reduced zones.

SECTION III

NITRATE REMOVAL FROM FLOODWATER OVERLYING FLOODED SOILS AND SEDIMENTS

Quantitative studies of the rates of floodwater nitrate (NO_3^-) removal by flooded soils and sediments are essential to an understanding of the capacity of wetland areas to act as a sink for NO_3^- polluted waters. Oxygen-deficient conditions, existing in continuously-flooded and seasonally flooded areas, help to govern the reactions, availability, or removal of several important sediment nutrients or pollutants. This is especially true for the nitrogen system as NO_3^- is readily reduced under oxygen-deficient conditions.

Microbial NO_3^- reduction, or denitrification, in a submerged sediment or soil, as a significant mechanism for removal of large amounts of soil nitrogen, is a well-known phenomenon. Losses of nitrogen by denitrification have been shown to range from 15 to 1,000 ppm for time intervals varying from 1 to 4 days. Patrick (1960) recorded a NO_3^- reduction rate of 15 ppm/day in a reduced soil and except where rates in excess of 1,000 ppm of NO_3^- were added, the NO_3^- reduction rate was a zero-order reaction with respect to NO_3^- concentrations.

The rate of denitrification in flooded soils or sediments may be affected by several mechanisms, some of which are: depth of overlying water, organic matter content of the soil, development of the aerobic-anaerobic zone at the mud-water interface, and diffusion to the zone of active denitrification. The profile differentiation is characterized by two distinct layers of soil or sediment: (1) a surface-oxidized layer of varying depth,

and (2) an underlying reduced layer (Pearsall 1950). The oxidation-reduction (redox) potential of an oxidized zone is generally higher than 300 mV and NO_3^- is usually considered to be relatively stable. However, in the anaerobic layer which is less than 300 mV, active and complete NO_3^- reduction will take place (Patrick and DeLaune 1972). Diffusion of NO_3^- to the active sites of denitrification plays an active role in controlling the rate of floodwater NO_3^- removal. The concentration gradient decreases in the direction of active denitrification sites and convection currents and perturbation by wind tends to keep the floodwater of these relatively shallow areas well-mixed. Soil organic matter content, as a microbial energy source, has a significant effect on the rate of NO_3^- reduction, as shown by the work of Bremner and Shaw (1958) in which 1,000 ppm NO_3^- was lost in 4 days from a submerged soil to which an energy source had been added.

The objectives of this study were to quantitatively characterize the floodwater NO_3^- removal capacity of two flooded areas and to differentiate between NO_3^- removal from the floodwater by diffusion alone, and NO_3^- removal by a combination of diffusion and NO_3^- reduction. The areas selected for this evaluation were the intermittently-flooded, fresh water swamp soils of the lower Mississippi River alluvial areas of Louisiana and the continuously-flooded salt water marsh soils of the Barataria Bay area of Louisiana. In addition, the effect of additions of an energy source on the thickness of the aerobic portion of the aerobic-anaerobic zone and its relationship to NO_3^- removal in Louisiana riceland soil was studied.

MATERIALS AND METHODS

Floodwater NO_3^- Removal by Two Flooded Soils

Three core samples each of a fresh water swamp soil (Bayou Sorrel, Louisiana) and salt water marsh soil (Barataria Bay, Louisiana) were obtained by driving thin wall aluminum pipes, 15.2 cm in diameter, into the soil to a depth of about 55 cm and removing them intact to the laboratory. The core samples were taken at sites about 150 m apart. The fresh water swamp soil was a mineral soil containing about 4% organic carbon and the salt water marsh soil was an organic soil containing about 12 to 14% organic carbon. The marsh soil is located in the intertidal zone of the Gulf of Mexico. The floodwater remained over the cores and additional floodwater was obtained for each core. Each core was sealed at the bottom and covered to prevent leakage and evaporation. The cores were then incubated in the laboratory at 30C while the additional water samples were stored at 4C. The floodwater of each core was then regulated to a depth of 7.6 cm and received 25 ppm NO_3^--N (KNO_3) which was evenly distributed throughout the floodwater. The NO_3^- removal capacity of these soils was then evaluated by a routine analysis of the floodwater for NO_3^- over a 10-day incubation period. This was accomplished by lowering a pipette exactly 2.5 cm from the water surface and withdrawing a 2-ml sample. The samples were frozen for later analysis by the phenoldisulfonic (PDS) acid method (Jackson 1958). To further explore the active site of NO_3^- removal, 1 liter of floodwater was removed from about 1 cm above the surface of each soil core, mixed with KNO_3 and sampled periodically for NO_3^-. To differentiate between NO_3^- diffusion from floodwater to soil alone

and by diffusion plus denitrification, the soil cores were sterilized with formaldehyde to give a 1.0% concentration throughout the core. The soil cores were then incubated for 7 days and the sterility checked. Potassium nitrate was then added to the cores as before and the floodwater sampled as previously described. Prior to NO_3^- analysis with PSD the formaldehyde was destroyed with 30% H_2O_2.

Floodwater NO_3^- Removal and Thickness of the Aerobic Layer as Affected by Addition of Organic Material

Crowley silt loam (a Louisiana riceland soil containing about 0.7% organic carbon) was mixed with varying amounts of finely-ground rice (Oryza sativa L.) straw to give quantities of soil containing 0.0, 0.1, 0.5, and 2.0% added organic matter (OM). Duplicate 400-g portions of the treated soils were weighed into 9 by 18 cm glass jars and flooded with 570 cc H_2O to a depth of 7.6 cm. The flooded soils were preincubated for 21 days to ensure reducing conditions and establishment of aerobic-anaerobic zones at the soil-water interface (Mortimore 1941). After preincubation, NO_3^- as KNO_3 was added to the floodwater as previously described. To characterize the aerobic-anaerobic layers of the treated soils, a redox profile of the soil-water interface to a depth of 20 mm was made as described by Patrick and DeLaune (1972). To differentiate NO_3 diffusion from microbial NO_3^- reduction the flooded soils were sterilized and treated as previously described.

To facilitate interpretation of the changes in floodwater NO_3^--N concentration, a quadratic regression analysis of each group of data from the various treatments was made. The data were calculated as the curve

most closely approximating a set of data points, $\underline{t}$ (time) and $\underline{y}$ (NO_3^--N concentration), expressed by the equation

$$y = at + bt^2 + c$$

with $\underline{a}$ as the initial rate of NO_3^- removal and $\underline{c}$ as the initial NO_3^- concentration. To differentiate diffusion of NO_3^--N from microbial NO_3^--N removal, the equation depicting NO_3^- loss in the sterile series was algebraically subtracted from the equation for NO_3^- loss in the nonsterile series. The resulting equation describes the rate of NO_3^- removal by microbial action only.

RESULTS AND DISCUSSION

Floodwater NO_3^- Removal by Two Flooded Soils

Floodwater NO_3^- removal rates by three undisturbed soil cores of the fresh water swamp of the Bayou Sorrel area of the lower Mississippi River alluvium are presented as rate curves in Figure 10. Equations representing the best fit curves are shown in Table 1. Total NO_3^- removal characterized by diffusion of NO_3^- and microbial NO_3^- reduction was relatively rapid with all NO_3^- disappearance in 10 days or less. The initial rates of total NO_3^- removal as underscored in Table 1 were 4.12, 4.03, and 4.98 ppm NO_3^--N for cores A, B, and C of the mineral soil. Decreases in NO_3^- concentration due to diffusion only are shown in Figure 10 for the sterile series and initial rates of diffusion are underscored in Table 1. Little variation was noted in the initial diffusion rates of cores A and B (1.61 and 1.62 ppm NO_3^--N), but the diffusion rate in core C was slightly higher (2.38 ppm NO_3^--N). The rate curves, representative of microbial NO_3^- removal only, are presented in the lower portion of Figure 10 and are

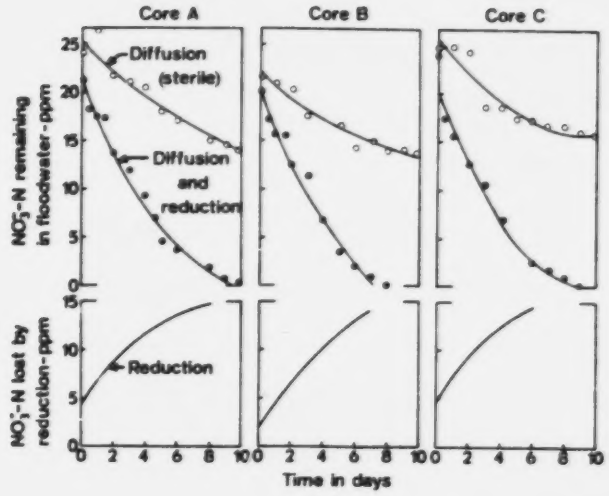


Figure 10. Floodwater NO_3^- removal in relatively undisturbed cores of a fresh water swamp soil as affected by diffusion and reduction.

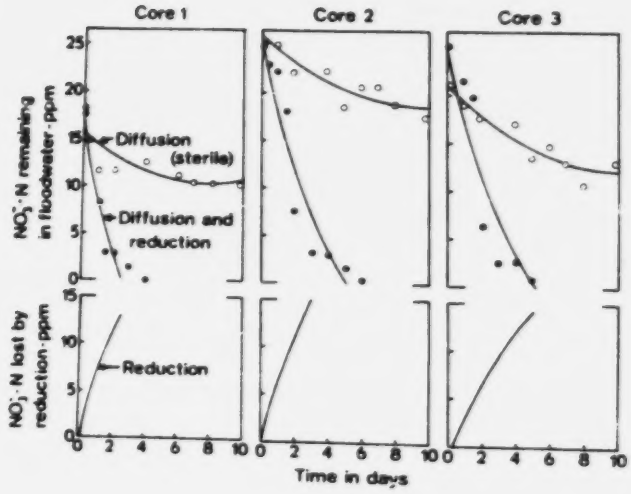


Figure 11. Floodwater NO_3^- removal in relatively undisturbed cores of a salt water marsh soil as affected by diffusion and reduction.

Table 1. QUADRATIC REGRESSION EQUATIONS AND CORRELATION COEFFICIENTS REPRESENTING THE RATES OF NITRATE REMOVAL FROM THE FLOODWATER OF FRESH WATER SWAMP SOILS AND SALT WATER MARSH SOILS FROM THE BAYOU SORREL AREA OF THE MISSISSIPPI RIVER ALLUVIUM AND BARATARIA BAY AREA OF LOUISIANA†

NO_3^--N removal by diffusion and reduction (nonsterile)	NO_3^--N removal by diffusion (sterile)	NO_3^--N removal by microbial reduction
	ppm/N	
	Bayou Sorrel, 7.6 cm H_2O Depth	
	Core A	
$y = \underline{-4.12t} + 0.20t^2 + 21.43$ $r = 0.996^{**}$	$y = \underline{-1.61t} + 0.05t^2 + 25.21$ $r = 0.980^{**}$	$y = 2.51t - 0.15t^2 + 3.78$
	Core B	
$y = \underline{-4.03t} + 0.18t^2 + 20.05$ $r = 0.993^{**}$	$y = \underline{-1.62t} + 0.08t^2 + 22.33$ $r = 0.984^{**}$	$y = 2.41t - 0.10t^2 + 2.28$
	Core C	
$y = \underline{-4.98t} + 0.29t^2 + 21.60$ 0.987^{**}	$y = \underline{-2.38t} + 0.13t^2 + 26.34$ 0.962^{**}	$y = 2.60t - 0.16t^2 + 4.74$

Table 1 continued.

Table 1 (continued): QUADRATIC REGRESSION EQUATIONS AND CORRELATION COEFFICIENTS REPRESENTING THE RATES OF NITRATE REMOVAL FROM THE FLOODWATER OF FRESH WATER SWAMP SOILS AND SALT WATER MARSH SOILS FROM THE BAYOU SORREL AREA OF THE MISSISSIPPI RIVER ALLUVIUM AND BARATARIA BAY AREA OF LOUISIANA†

NO_3^--N removal by diffusion and reduction (nonsterile)	NO_3^--N removal by diffusion (sterile)	NO_3^--N removal by microbial reduction
ppm/N		
	Barataria Bay 7.6 cm H_2O Depth	
	Core 1	
$y = -10.60t + 1.46t^2 + 17.4$ $r = 0.969^{**}$	$y = -1.24t + 0.08t^2 + 15.39$ $r = 0.737^{**}$	$y = 9.36t - 1.38t^2 - 2.01$
	Core 2	
$y = -8.92t + 0.75t^2 + 26.6$ 0.969^{**}	$y = -1.34t + 0.06t^2 + 25.7$ $r = 0.858^{**}$	$y = 7.58t - 0.69t^2 - 0.9$
	Core 3	
$y = -7.92t + 0.61t^2 + 25.0$ 0.948^{**}	$y = -1.72t + 0.08t^2 + 2.09$ $r = 0.949^{**}$	$y = 6.20t - 0.53t^2 - 4.1$

** Significant at the 1.0% level of statistical probability.

† The curve most closely approximating a set of data points where t is time in days and y is ppm NO_3^--N and is expressed by the equation $y = at + bt^2 + C$ where r is the correlation coefficient.

the algebraic differences of the two upper curves. The regression equations for these curves are shown in Table 1. The initial rates of microbial NO_3^- removal were similar for the three cores (2.51, 2.41, and 2.60 ppm NO_3^--N). These data demonstrate a rapid movement of NO_3^- out of the relatively shallow floodwater of these soils with most NO_3^- disappearance attributed to microbial removal. However, when the floodwater was incubated out of contact of the soil there was no decrease in the NO_3^- concentration of the water showing that there was no active microbial NO_3^- removal in the water.

Total NO_3^- removal from the saline floodwater overlying the organic marsh soils of Barataria Bay was very rapid, as shown by the rate curves in Figure 11, with complete disappearance of NO_3^- after 6 days. Rates of initial NO_3^- removal were calculated and are underscored in Table 1. These rates were 10.60, 8.92, and 7.92 ppm NO_3^--N for cores 1, 2, and 3. As contrasted to the swamp soil, the rate of total NO_3^- removal was much higher; however, a greater variation in rate was noted among the marsh cores. The rate curves for NO_3^- diffusion are also presented in Figure 11 with the initial rate of NO_3^- diffusion underscored in Table 1. These rates were 1.24, 1.34, and 1.72 ppm NO_3^--N for cores 1, 2, and 3 and were similar to diffusion rates calculated for the fresh water cores. Consequently, the calculated rates of microbial NO_3^- reduction were appreciably faster than those for the fresh water soil and were 9.36, 7.36, and 6.20 ppm NO_3^--N for cores 1, 2, and 3, respectively. These data showed a very active microbial NO_3^- removal demonstrating that these marsh soils can act as a sink for significant amounts of NO_3^-. As with the fresh water soil, no NO_3^- was reduced in floodwater separated from the marsh soil cores.

Floodwater NO_3^- Removal and Thickness of the Aerobic Layer as Affected by Addition of Organic Material

The effects of additions of various amounts of organic matter as ground rice straw on the thickness of the aerobic part of the aerobic-anaerobic zone and on NO_3^- removal rate from the overlying floodwater were studied on Crowley silt loam, a riceland soil relatively low in native organic matter. The increasing additions of organic material greatly increased microbial activity, consequently resulting in thinner aerobic layers at the soil-water interface. The redox profile curves used to characterize the aerobic-anaerobic double layer to a depth of 20 mm are shown in Figure 12. The depth of the aerobic zone (redox potential greater than 300 mV) extended 14 mm from the soil surface for the untreated soil; a sharp decrease in depth occurred with increased additions of organic matter. The thickness of the aerobic layers of soil were 11 and 2.5 mm for the 0.1 and 0.5 additions of rice straw. For the 2.0% addition, reducing conditions extended to the soil surface with little detectable aerobic zone. Rice straw additions had two effects on NO_3^- reduction; the thinner aerobic zone decreased the distance over which NO_3^- diffused to the site of denitrification, the anaerobic soil layer, and the added energy source increased the total capacity for NO_3^- reduction. It appears from the redox profile of the soil receiving the 2.0% addition of rice straw that NO_3^- would be actively denitrified at or very near the soil-water interface. Consequently, there would be no dilution of NO_3^- by diffusion into the soil. The increased microbial activity placed a greater consumptive demand on free oxygen diffusing through the floodwater to the soil-water interface and in the case of the 2.0%

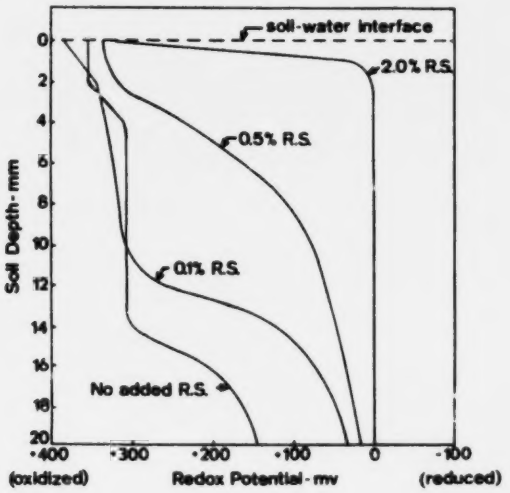


Figure 12. The effects of additions of rice straw on the development of redox potential profiles in a flooded rice-land soil. R.S. = rice straw.

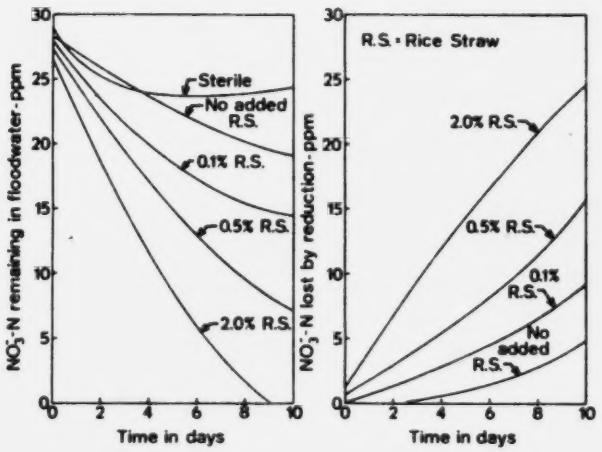


Figure 13. The effects of additions of rice straw on the removal of floodwater NO_3^- by diffusion and reduction. R.S. = rice straw.

level of rice straw the free oxygen appeared to be consumed at the soil-water interface and did not move into the soil. The effects of the additions of rice straw on NO_3^- removal rates are presented in Figure 13 and calculated rates are shown and underscored in Table 2. The initial rates of total NO_3^- removal were drastically affected by the addition of the energy source and were 1.47, 2.60, 2.67, and 4.79 ppm NO_3^--N per day for the 0, 0.1, 0.5, and 2.0 additions. Nitrate removal due to diffusion is shown by the curve for the sterile series. Diffusion of NO_3^- was similar for all rice straw treatments and is presented as an average for all treatments. Microbial NO_3^- reduction appeared to be the dominant removal mechanism, especially after the first few days.

The rates of microbial NO_3^- reduction as the difference of the sterile and nonsterile curves are also presented in Figure 13. These curves depict a sharp increase in NO_3^- reduction with increasing organic matter. The initial rates of NO_3^- reduction, as underscored in Table 2, were -0.17, 0.96, 1.03, and 3.15 ppm NO_3^--N per day for the 0, 0.1, 0.5, and 2.0% additions of rice straw, respectively. The negative value was due to the fact that microbial reduction plus diffusion was not appreciably greater than diffusion alone for several days in the untreated soil.

CONCLUSIONS

This study has shown that both fresh water swamp and salt water marsh soils of Louisiana can remove NO_3^- rapidly from the overlying floodwater. Of the two kinds of soil studied, the salt water marsh was the most effective sink for NO_3^- with an average removal rate of 9.15 ppm N/day. Converting these data to a hectare basis would result in a NO_3^- removal

Table 2. QUADRATIC REGRESSION EQUATIONS AND CORRELATION COEFFICIENTS REPRESENTING THE RATES OF NITRATE REMOVAL FROM THE FLOODWATER OF FLOODED CROWLEY SILT LOAM SOILS†

Organic matter addition	NO_3^--N removal by diffusion and reduction (nonsterile)	NO_3^--N removal by diffusion (sterile)	NO_3^--N removal by microbial reduction
0.0%	$y = -1.47t + 0.05t^2 + 28.1$ $r = 0.963^{**}$	$y = -1.64t + 0.12t^2 + 28.1$ $r = 0.934^{**}$	$y = -0.17t + 0.07t^2 + 0$
0.1%	$y = -2.60t + 0.11t^2 + 28.5$ $r = 0.988^{**}$		$y = 0.96t + 0.01t^2 - 0.4$
0.5%	$y = -2.67t + 0.07t^2 + 27.6$ $r = 0.983^{**}$		$y = 1.03t + 0.05t^2 + 0.5$
2.0%	$y = -4.79t + 0.21t^2 + 26.5$ $r = 0.994^{**}$		$y = 3.15t + 0.09t^2 + 1.16$

** Significant at the 1.0% level of statistical probability.

† The curve most closely approximating a set of data points where t is time in days and y is ppm NO_3^--N and is expressed by the equation $y = at + bt^2 + C$ where r is the correlation coefficient.

rate of about 7.4 kg N/ha per day. The fresh water swamp, also an effective sink for NO_3^-, had an average removal rate of 3.5 kg N/ha per day, about one-half that of the salt water marsh. Further studies showed that additions of organic matter as ground rice straw regulated the thickness of the aerobic-anaerobic double layer of a flooded soil and drastically affected the rate of floodwater NO_3^- removal. The soil receiving the largest addition of organic matter had the highest rate of NO_3^- removal. This effect was due to increased microbial activity which caused a thinner aerobic-anaerobic zone and in turn decreased the distance over which NO_3^- could diffuse to the underlying anaerobic zone and increased the NO_3^- reduction capacity of the anaerobic zone.

SECTION IV

THE ROLE OF OXYGEN IN NITROGEN LOSS FROM FLOODED SOILS

Recent experiments utilizing ^{15}N as a tracer have shown that N loss from flooded soils occurs only when the flooded soil is exposed to O_2 (Tusneem and Patrick 1971; Patrick and Tusneem 1972; Broadbent and Tusneem 1971). The development of a thin oxygenated surface layer of soil permits the biological oxidation of ammonium N to nitrate which in turn diffuses into the underlying anaerobic layer where denitrification occurs. Although the experiments cited above showed the effect of exposing the flooded soil to air containing 21 percent O_2, no information is available on the effect of different concentrations of O_2 on the magnitude of N loss. This study deals with the loss of labeled N from flooded soil incubated under N_2-O_2 mixtures containing various concentrations of O_2. The effect of O_2 content on the thickness of the surface aerobic soil layer where nitrification occurs was also measured.

MATERIALS AND METHODS

Crowley silt loam soil with an initial total N content of 0.087 percent was sampled from the plow layer of a field used for lowland rice, dried, and passed through a 40-mesh sieve. The dry soil was tumble-mixed for several hours with 230 ppm N as $(NH_4)_2SO_4$ which contained 10.228 atom percent ^{15}N excess. Duplicate 100-g samples of the soil were weighed into 8-oz. wide-mouth glass bottles and flooded with an equal weight of water. The depth of soil in the bottle was 4 cm with 2.7 cm of overlying flood water.

Incubation was carried out in the dark at 30°C for periods up to 120 days under O_2-N_2 mixtures containing 0, 5, 10, 20, 40, and 80 percent O_2. The bottles were incubated in the dark in large sealed containers through which flowed a slow stream of moist air of the desired O_2 content. Samples were analyzed in quadruplicate periodically for total N (labeled plus unlabeled) in both the inorganic (nitrate + nitrite + ammonium) fraction and the organic fraction (Bremner 1965).

The thickness of the aerobic surface layer of soil was determined by a method described earlier (Patrick and DeLaune 1972) in which the redox potential profile was measured by advancing a slow-moving platinum electrode downward through the flooded soil.

RESULTS AND DISCUSSION

The magnitude of N loss was governed by the amount of O_2 in the atmosphere over the flooded soil. Losses of both total N (Figure 14) and labeled N (Figure 15) increased as the O_2 content increased. Maximum losses of approximately 183 ppm total N (17 percent of total) and 155 ppm labeled N (67 percent of that added) at 120 days were recorded. Most of the N loss occurred at the expense of the inorganic fraction (which was present almost entirely as added labeled N). A small amount of labeled N entered and remained in the organic fraction. Little loss of N occurred at zero O_2. The small amount lost at zero O_2 was probably the nitrate present at the beginning of incubation. Nitrogen loss increased greatly with small increases in O_2 content. An increase in O_2 content above 20 percent had little additional effect on N loss. Apparently the O_2 content of the earth's atmosphere is sufficient to cause near-maximum denitrification loss of N in flooded soil.

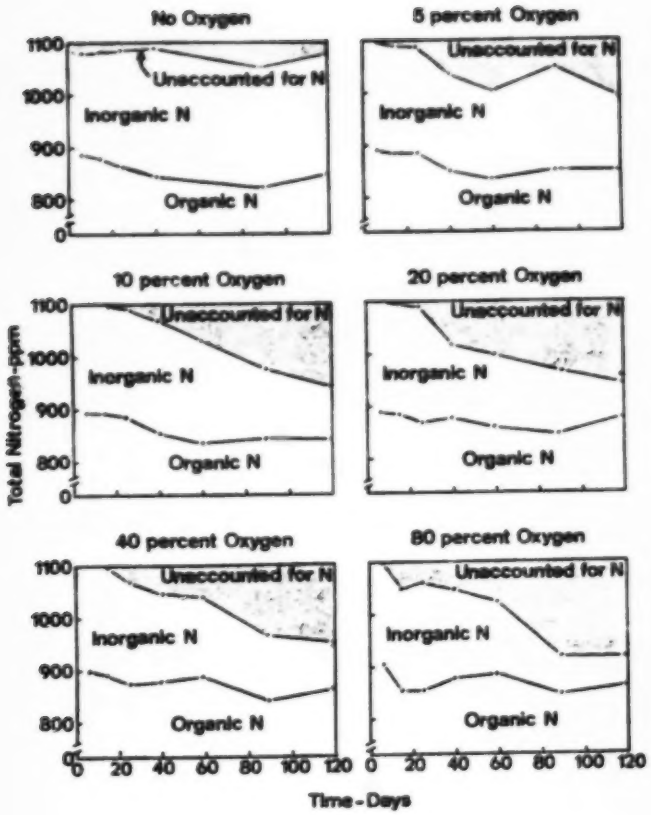


Figure 14. Loss of total N (labeled + unlabeled) from flooded soils as affected by O_2 content of air.

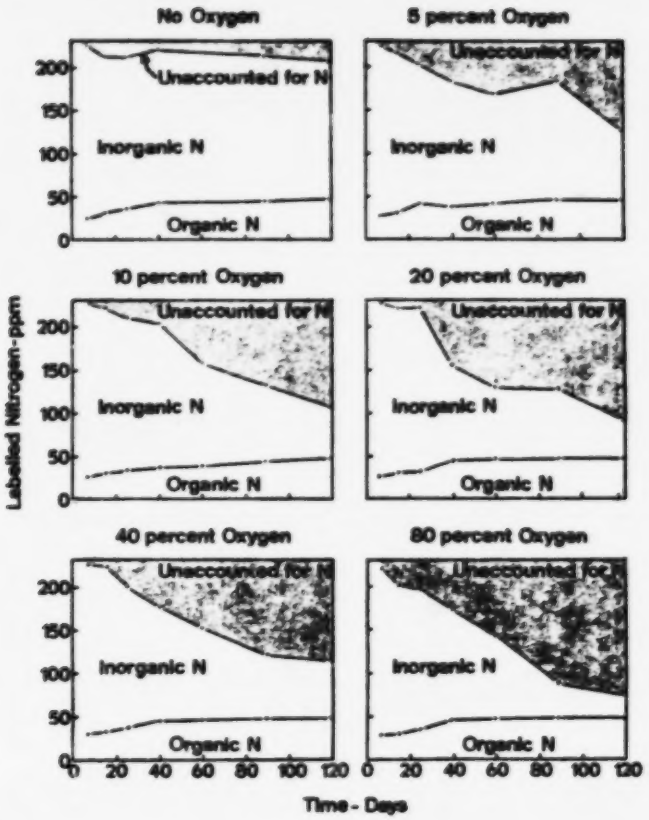


Figure 15. Loss of labeled N from flooded soil as affected by O_2 content of air.

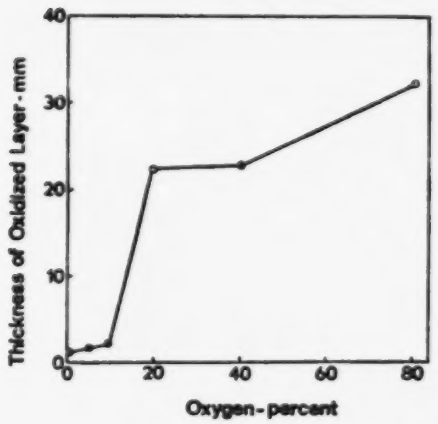


Figure 16. Effect of O_2 content of air on thickness of aerobic soil layer after 90 days' incubation.

Because of the role of the aerobic soil layer in supporting nitrification, a study was made of the effect of O_2 content on the thickness of this oxygenated layer, since a previous experiment (Patrick and Tusneem 1972) indicated that a thick aerobic layer would result in higher N loss than a thin layer. The thickness of the aerobic layer at the various O_2 levels after 90 days of incubation is shown in Figure 16. The first increments of O_2 (up to 10 percent) created only a thin aerobic layer while O_2 contents of 20 percent and higher resulted in a layer 2 to 3 cm thick. The extent of N loss did not correspond precisely to the thickness of the aerobic layer since appreciable loss occurred at 5 and 10 percent O_2 where only a thin aerobic layer existed. Although the thickness of the aerobic layer is important in helping to determine the amount of nitrate formed from ammonium, it is not the only factor governing this process. The rate of ammonium diffusion from the underlying reduced soil to the aerobic surface layer, regardless of how thin this layer is, contributes to the amount of nitrate formed.

Because of the known effect of organic matter in immobilizing N in an organic form not subject to denitrification, an additional treatment was included in which the soil received 0.5 percent ground rice straw and was incubated for 120 days under the various O_2 concentrations. The results obtained (Table 3) show an appreciable conservation of N as a result of added organic matter, with N loss being decreased 30 to 40 ppm labeled N and slightly more total N. Analyses of inorganic and organic N fractions showed that the rice straw immobilized an appreciable amount of the inorganic N, thereby protecting it from nitrification and subsequent denitrification.

Table 3. NITROGEN CONTENT OF FLOODED SOIL IN PPM AFTER 120 DAYS' INCUBATION WITH AND WITHOUT ADDITION OF 0.5 PERCENT RICE STRAW (FOUR REPLICATIONS)

Oxygen %	Total N		Labelled N	
	Rice Straw	No rice straw	Rice straw	No rice straw
0	1081	1077	196	207
5	1049	994	159	127
10	1018	944	138	107
20	1016	947	140	92
40	1056	951	153	117
80	957	920	104	76

The results of this study show that the amount of O_2 over a flooded soil is a factor in determining the amount of N lost by denitrification. Large increases in N loss occurred from the first few increments of oxygen with little further loss occurring above 20 percent O_2. The thickness of the aerobic soil layer was also governed by the amount of O_2 in the air. Nitrogen loss was generally related to the thickness of the aerobic layer, even though appreciable loss occurred at 5 and 10 percent O_2 where the aerobic layer was relatively thin. Ammonium diffusion from the underlying anaerobic zone to the aerobic surface layer is probably a significant factor in determining the rate of N loss in flooded soils. Adding organic matter to the soil significantly reduced N loss.

SECTION V

NITRIFICATION-DENITRIFICATION REACTIONS IN FLOODED SOILS AND SEDIMENTS: DEPENDENCE OF OXYGEN SUPPLY AND AMMONIUM DIFFUSION

Denitrification in soils, swamps, marshes and water bodies is the major process by which elemental nitrogen is returned to the atmosphere. Denitrification takes place when a deficiency of oxygen causes certain facultative anaerobic bacteria to use nitrate in place of oxygen as an electron acceptor for respiration. Although denitrification in soils as a result of temporary anaerobic conditions caused by variations in soil moisture has been recognized for years (Russell, 1961), the extent of and mechanisms involved in nitrogen loss in continuously flooded systems are not as well documented.

Ammonium is the predominant inorganic form of nitrogen in oxygen-deficient flooded systems and denitrification cannot take place unless conditions exist where nitrification of the ammonium can first occur. The development of a thin oxygenated surface layer as a result of dissolved oxygen penetrating a short distance into the flooded soil or sediment before being consumed allows the oxidation of ammonium to nitrate to take place in this oxygenated or aerobic surface layer. Nitrate produced from this reaction diffuses down into the underlying oxygen-free layer where it is denitrified.

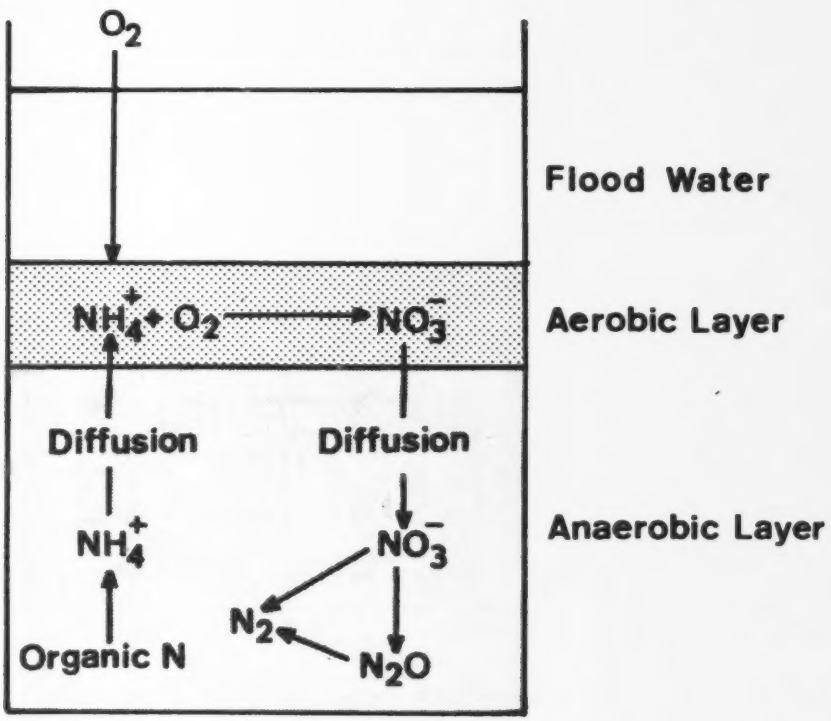
Recent experiments have shown that appreciable denitrification will occur in flooded soils if both oxygen from the atmosphere and ammonium from the flooded soil are available (Tusneem and Patrick, 1971; Broadbent and Tusneem, 1971). Although the nitrogen converted from nitrate to gaseous

forms in flooded systems is derived from ammonium oxidized to nitrate in the aerobic layer, the amount of nitrogen gas usually greatly exceeds the amounts of ammonium and nitrate present in the aerobic layer at any one time. Ammonium movement from the underlying anaerobic layer to the surface aerobic layer is apparently necessary to account for the large nitrogen losses that occur in flooded systems. Removal of ammonium by nitrification in the aerobic layer creates a concentration gradient which causes ammonium in the underlying anaerobic layer to diffuse upward to the aerobic layer where it undergoes nitrification. Nitrate formed by this process readily diffuses down toward the nitrate-free anaerobic layer where it is denitrified to nitrogen gas by serving as an electron acceptor in the oxidation of organic matter. These processes are illustrated in Figure 17. As indicated in the equation, nitrate is an intermediate in the overall pathway between ammonium and elemental nitrogen.

In the experiments described in this report, the processes discussed above for governing nitrogen loss in flooded soils and sediments are examined. Particular attention is given to the roles of oxygen supply to the aerobic layer and ammonium diffusion from the anaerobic layer to the aerobic layer in determining nitrogen loss through the nitrification-denitrification process. Nitrogen-15 labeled ammonium was used to trace nitrogen through the various nitrogen forms.

MATERIALS AND METHODS

The experiment was carried out using large (45.5 cm length x 4.5 cm diameter) pyrex tubes fitted at the top with a stopcock and a ground glass junction for entry to a mass spectrometer. All connections in the apparatus



Nitrification – Denitrification Reaction:

$$24\,NH_4^+ + 48\,O_2 \longrightarrow 24\,NO_3^- + 24\,H_2O + 48\,H^+$$

$$24\,NO_3^- + 5\,C_6H_{12}O_6 + 24\,H^+ \longrightarrow 12\,N_2 + 30\,CO_2 + 42\,H_2O$$

$$24\,NH_4^+ + 5\,C_6H_{12}O_6 + 48\,O_2 \longrightarrow 12\,N_2 + 30\,CO_2 + 66\,H_2O + 24\,H^+$$

Figure 17. Processes involved in the sequential conversion of organic nitrogen to elemental nitrogen in flooded soils and sediments.

were ground glass and tested for leaks. Fifty grams of soil (Crowley silt loam) and enough water (50 ml) to provide a 1.5 cm water layer over the soil were added to each flask. Ammonium sulfate with an ^{15}N enrichment of 29.944 atom-% was thoroughly mixed with the soil at the rate of 250 µg N per g soil. The effect of oxygen in the atmosphere over the flooded soil on the conversion of ammonium nitrogen to nitrogen gas was studied in two experiments. In one experiment, an atmosphere of 30% oxygen and 70% argon was maintained over the flooded soil. Duplicate flasks were removed after various periods of incubation for analyses of five labeled nitrogen fractions: ammonium, organic (protein) nitrogen, nitrate, nitrous oxide, and elemental nitrogen. The amount of labeled nitrogen was determined in each of these fractions by appropriate procedures employing a mass spectrometer (Bremner, 1965). In the other experiment, conditions were the same as described above except that variable oxygen contents ranging from 0 to 50% were maintained over the flooded soil for 100 days, after which the distribution of labeled nitrogen was determined. Side tubes containing ascarite granules were placed in each flask to absorb excess carbon dioxide. The amount of oxygen consumed was also determined by mass spectrometer analysis. The size of the flask and the amount of soil were chosen so that large changes in the oxygen content did not occur during incubation.

Separate experiments were carried out to measure the magnitude of ammonium diffusion from the anaerobic layer to the aerobic surface layer. One method consisted of incubating columns of flooded soil containing 200 µg ammonium N per g soil and determining ammonium distribution by slicing the soil columns horizontally into 2 mm sections. The distribution curves

were used to calculate the ammonium diffusion from the anaerobic layer to the aerobic layer by measuring the decrease in ammonium in the anaerobic layer with time. Another approach to the estimation of ammonium diffusion from the anaerobic layer into the aerobic layer was to measure the total amount of ammonium converted to nitrogen gas and nitrate and to subtract from this value the amount of ammonium originally present in the aerobic layer. Diffusion was not involved in the nitrification of ammonium originally present in the aerobic layer. In order to use this method it was necessary to allow the reaction to proceed long enough so that all of the ammonium originally present in the aerobic layer had been converted to nitrate. For both the above methods, it was also necessary to accurately determine the thickness of the reddish-brown oxidized ferric oxide layer overlying the grayish-brown ferrous oxide layer. The thickness of the aerobic layer determined by this method was found to correspond closely with the thickness of the nitrate-containing layer.

RESULTS AND DISCUSSION

Results of the experiments dealing with the effect of oxygen on ammonium conversion to nitrate and then to nitrogen gas are shown in Figure 18. The ammonium content of the system decreased with time with a buildup of nitrogen gas evident after two weeks. No nitrous oxide (N_2O) was present until the last sampling at 100 days. Nitrate was present after 30 days, but did not accumulate. The most striking result of this experiment was the rapid conversion of ammonium to nitrogen gas after 30 days. This length of time was approximately the same as that required for the development of a pronounced aerobic surface layer in the flooded soil. A small

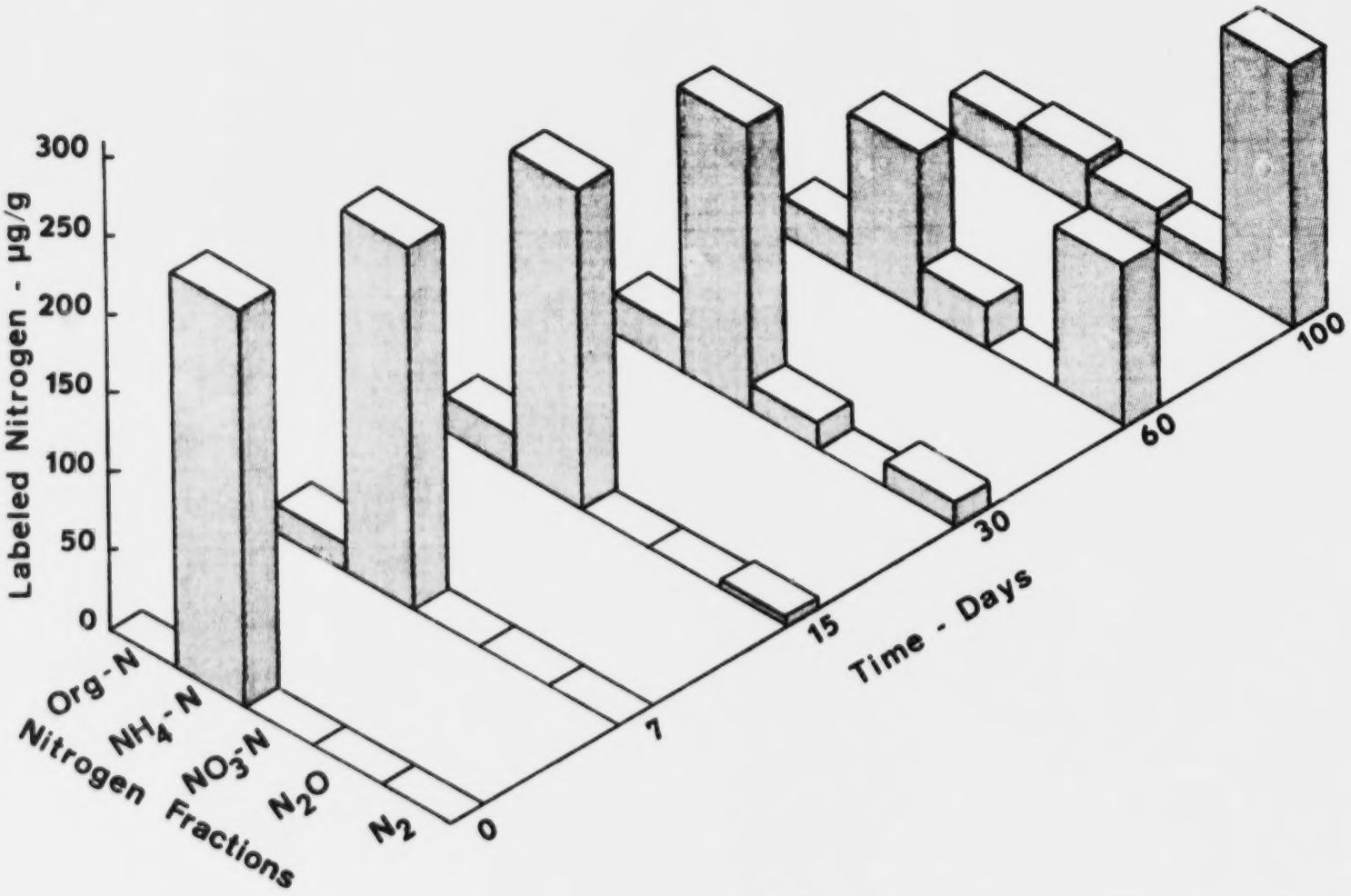


Figure 18a. The conversion of ^{15}N-labeled ammonium to elemental nitrogen in a flooded soil. The distribution of labeled ammonium among various nitrogen fractions following incubation under 30% oxygen.

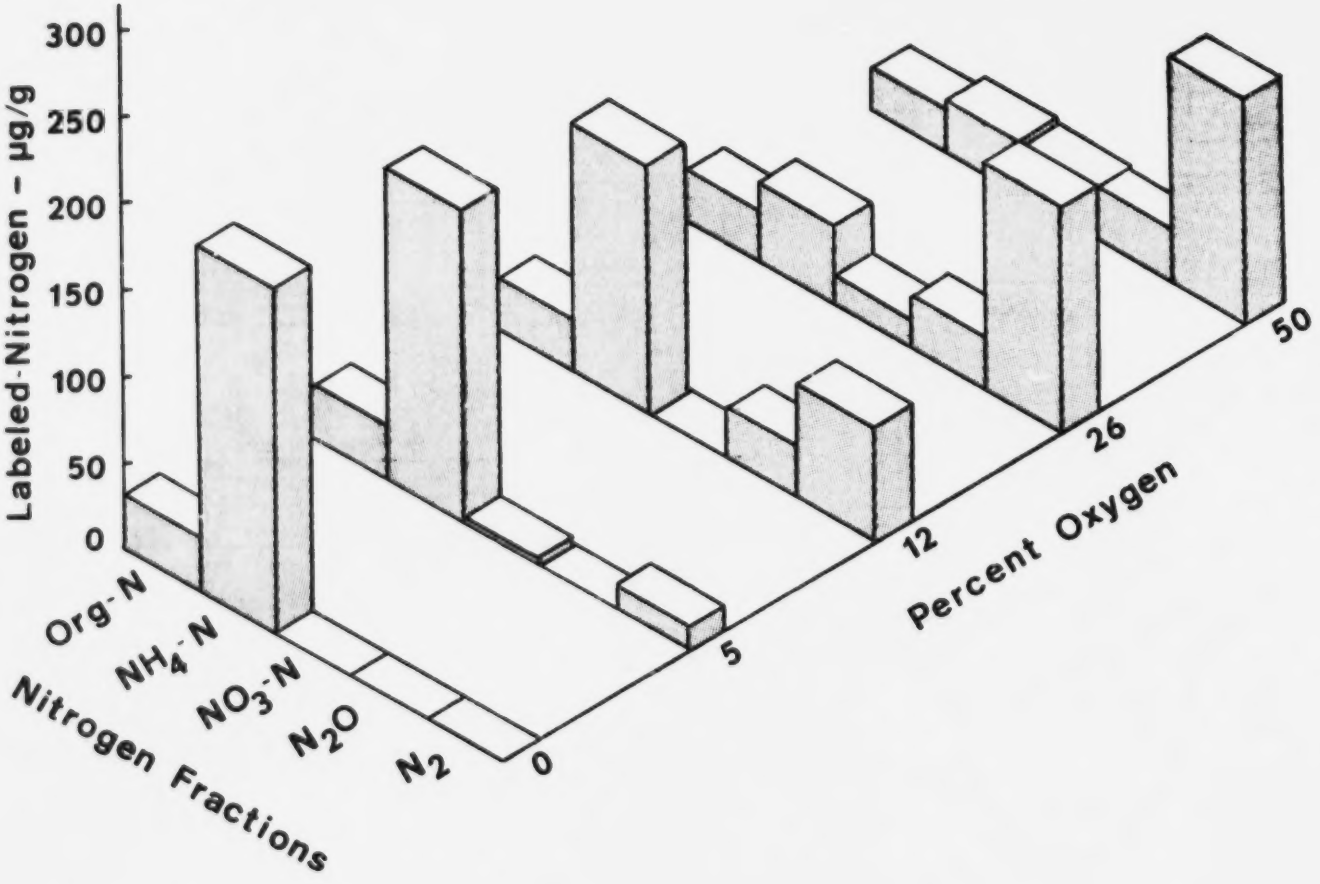


Figure 18b. The conversion of ^{15}N-labeled ammonium to elemental nitrogen in a flooded soil. The effect of oxygen content on conversion of labeled ammonium to various nitrogen fractions.

amount of labeled ammonium nitrogen was incorporated into the organic fraction at the beginning of incubation and did not change appreciably in concentration during the 100-day period.

The oxygen content of the atmosphere over the flooded soil during the 100-day incubation period had a marked effect on the stability of ammonium. Where no oxygen was present almost all of the added labeled ammonium remained in the ammonium form except for the small amount incorporated into the organic fraction. Where oxygen was present, however, at least part of the ammonium was converted to nitrogen gas, with the amount increasing as the oxygen content increased up to 26%. A higher oxygen content resulted in a thicker aerobic layer and slightly more nitrate, but no additional nitrogen gas.

These experiments utilizing tracer nitrogen demonstrate that ammonium in a flooded soil is converted to nitrate and then to elemental nitrogen. Measurements of the thickness of the aerobic surface layer and of the amount of labeled nitrogen gas produced showed that approximately twice as much ammonium was converted to nitrate and elemental nitrogen as was originally present in the aerobic layer. Calculation of ammonium diffusion from the anaerobic layer to the aerobic layer based on direct measurement of ammonium distribution with depth throughout the aerobic-anaerobic layers also showed that approximately half of the ammonium nitrogen involved (720 $\mu g\ cm^{-2}$ or 72 $kg\ ha^{-1}$) was derived from ammonium diffusing from the anaerobic layer to the aerobic layer. The measured diffusion coefficient for ammonium was $2.5 \times 10^{-6}\ cm^2\ sec^{-1}$ as determined by the method of Phillips and Brown (1964). Similarly, the diffusion coefficient for nitrate diffusing from the aerobic layer to the anaerobic layer was 1.5×10^{-5}

cm^2 sec^{-1}. These results are applicable for flooded soil or sediment systems in which no downward percolation occurs that could prevent upward diffusion of ammonium. In most flooded soils and shallow water bodies, percolation of water is extremely slow.

Oxygen consumption in the flooded soil is shown in Figure 19. Most of the oxygen consumed initially was probably used for oxidation of organic carbon by heterotrophs, but after several days a considerable portion was also being used for nitrification. Some of this oxygen was present as nitrate, while some had been converted from nitrate to carbon dioxide in the denitrification reaction (see equation in Figure 17). Increasing the concentration of oxygen over the flooded soil increased the thickness of the aerobic layer and increased the amount of oxygen consumed. Nitrification and organic carbon oxidation accounted for approximately equal amounts of oxygen.

CONCLUSIONS

The results of this study show that ammonium nitrogen in an anaerobic soil or sediment exposed to atmospheric oxygen undergoes sequential nitrification and denitrification. The source of the ammonium reacting with atmospheric oxygen in the aerobic layer consists of the ammonium initially present in the aerobic surface layer of soil or sediment plus an approximately equal amount diffusing to the aerobic layer from the underlying anaerobic layer. It is likely that a significant part of the ammonium present in flooded soils and shallow water sediments follows this pathway and makes a major contribution to the elemental nitrogen being returned to the atmosphere.

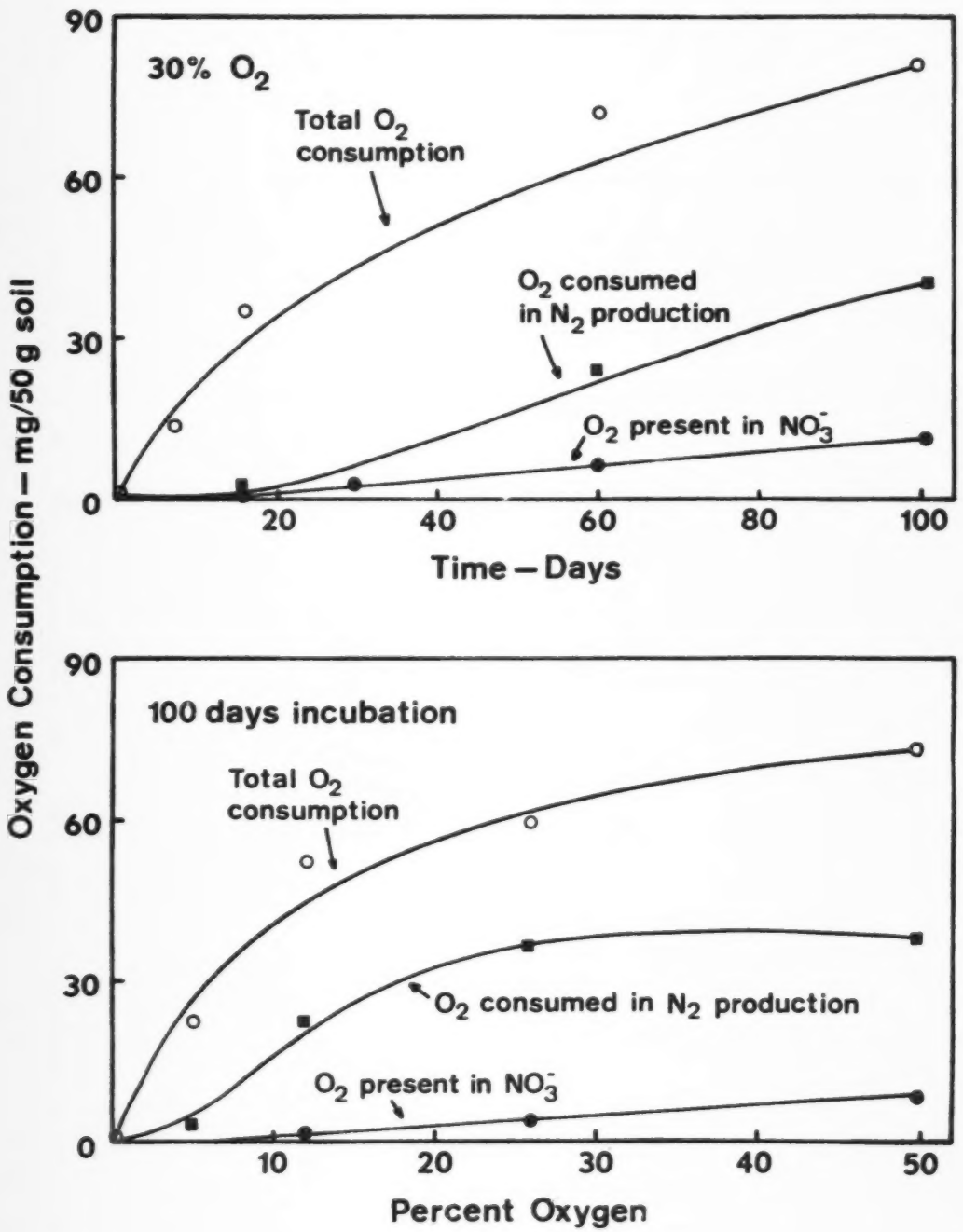


Figure 19. Total oxygen consumption, oxygen converted to nitrate, and oxygen converted sequentially to nitrate and then to carbon dioxide (see equation in Figure 17) as affected by time of incubation and oxygen concentration.

SECTION VI

EFFECT OF DISSOLVED OXYGEN ON REDOX POTENTIAL AND NITRATE REDUCTION IN FLOODED SWAMP AND MARSH SOILS

The NO_3^--N content of some natural bodies of surface water has increased slightly in recent years. This increase has been attributed to a number of sources, including the use of N- fertilizers for field crops and the surface disposal of N-containing wastes. The increase in NO_3^--N content of surface water supplies, part of the pollution load, has made it necessary to learn more about the mechanisms by which N is returned from the water to the atmosphere. In low lying swamp and marsh areas that receive local drainage from agricultural areas where large amounts of N- fertilizers are used, NO_3^--N is rapidly removed from the water through denitrification after the NO_3^--N has diffused through the interface separating the floodwater from the anaerobic mud layer (Engler and Patrick, 1974).

An anaerobic zone exists in a flooded soil because O_2 moving from the atmosphere through the floodwater can penetrate only a short distance in the soil before being utilized by aerobic and facultative anaerobic microorganisms. The layer below the zone of O_2 penetration becomes anaerobic rapidly after flooding (Patrick and DeLaune, 1972). In the anaerobic layer, many facultative anaerobic bacteria use NO_3^--N as a substitute for O_2, reducing the NO_3^--N to N_2 gas or N_2O which returns to the atmosphere (Pearsall, 1950; Mitsui, 1954). The reduction process is accentuated where there is an ample supply of organic detritus or energy source in the mud for bacterial metabolism. Although this mechanism of nitrogen

loss has been known to exist, little information is available on the effect of dissolved O_2 in the overlying and pore water on the process.

At the surface oxygenated sediment-water or soil-water interface, microbial and chemical conditions are very much like those in drained or oxidized soil. In the underlying O_2-free soil or sediment layer anaerobic biological and chemical transformations take place in the absence of O_2. In the presence of O_2 the oxidized constituents of the soil such as NO_3^--N, Mn^{4+} compounds, and Fe^{3+} compounds are not biologically or chemically reduced (Mortimer, 1941). After O_2 disappears following flooding of an oxygenated soil or sediment many soil micro-organisms are able to substitute one or more of these oxidized chemical components for the O_2 required in respiration. Nitrate, the higher oxides of Mn and hydrated ferric oxide are reduced more or less sequentially if O_2 becomes absent or limiting and if an energy source (organic matter) is available to the micro-organisms (Turner and Patrick, 1968). Nitrate and Mn^{4+} compounds are readily reduced since the energy required for their reduction is low and a number of species of micro-organisms are capable of carrying out this process.

The objectives of this study were to measure O_2 reduction rates, NO_3^- reduction rates, and the effects of added O_2 on NO_3^- reduction and redox potential in four swamp and marsh soils from Louisiana. In addition, laboratory experiments were set up to determine the site of NO_3^- reduction in these systems and to evaluate the role of NO_3^- diffusion from the overlying water into the reduced soil in the overall NO_3^- reduction process.

MATERIALS AND METHODS

Four soils taken from Mississippi River floodplain swamps and the Coastal Marsh of Louisiana were selected for this investigation. Both fresh and brackish water areas were represented. Bulk samples of soil were placed in several layers of large polyethylene bags, sealed to prevent moisture loss and minimize oxygen contact, and iced to reduce biological activity. Floodwater overlying the soils was collected in separate containers and both soils and floodwater were stored at 2 to 4°C until used in the experiment. A brief description of the four soils is given in Table 4. All soils were from areas subject to flooding all or part of the year. Selected chemical properties of these soils are presented in Table 5. Experiments were conducted at 30°C.

O_2 Removal from Soil Suspensions

Suspensions (soil:water = 1:2) of each of the four soils were placed in the flask shown in Figure 20 and stirred continuously. The container was completely filled so that no air space existed. Oxygen gas was bubbled through the suspension until the dissolved O_2 content as measured with a membrane-covered polarographic O_2 electrode increased to 16 ppm (on a water basis). The electrode was standardized with air saturated distilled H_2O at 30°C. The flask was sealed and both dissolved O_2 and redox potential were monitored continuously for several hours. Redox potential measurements were made with a bright platinum electrode and calomel half cell connected to a vacuum tube voltmeter. The filled flasks contained 1850 ml of suspension.

Table 4

DESCRIPTION OF SOILS USED IN STUDY

Soil No.	Description
1	This is an unclassified clay from a marsh that is intermediate between brackish and salt water. This soil is flooded throughout the year.
2	This soil is a muck taken from a fresh water swamp and consists of an organic surface horizon 4 to 15 inches thick underlain by a soft clayey horizon. This soil belongs to the Barberry series.
3	This soil is a muck from a slightly saline marsh with surface layers of well decomposed plant materials underlain by four feet of soft clay. The sample is of the Lafitte series.
4	This is a clay that is subject to flooding by salt water but which was unflooded at the time of sampling and belongs to the Lafitte series.
Control	This is a Crowley silt loam, a Louisiana Coastal Prairie soil used extensively for rice cultivation and containing about 0.7% organic carbon.

Table 5

CERTAIN CHEMICAL PROPERTIES OF SOILS USED IN THIS STUDY

Soil No.	pH	Moisture**	Organic Carbon	Total Sulfide	Extractable† P	Extractable† K	Extractable† Ca	Extractable† Mg
		percent		parts per million				
1	7.6	236.1	4.9	965.3	267	616	4400	1946
2	5.7	219.7	8.4	0.0	145	368	4400	1424
3	6.3	257.7	35.9	0.0	139	624	7200	6280
4	5.7	114.2	4.3	0.0	164	616	640	1070

** Grams of water per 100 grams of soil.

† P extracted with 0.1N HCl + 0.03N NH_4F; other elements extracted with 0.1N HCl.

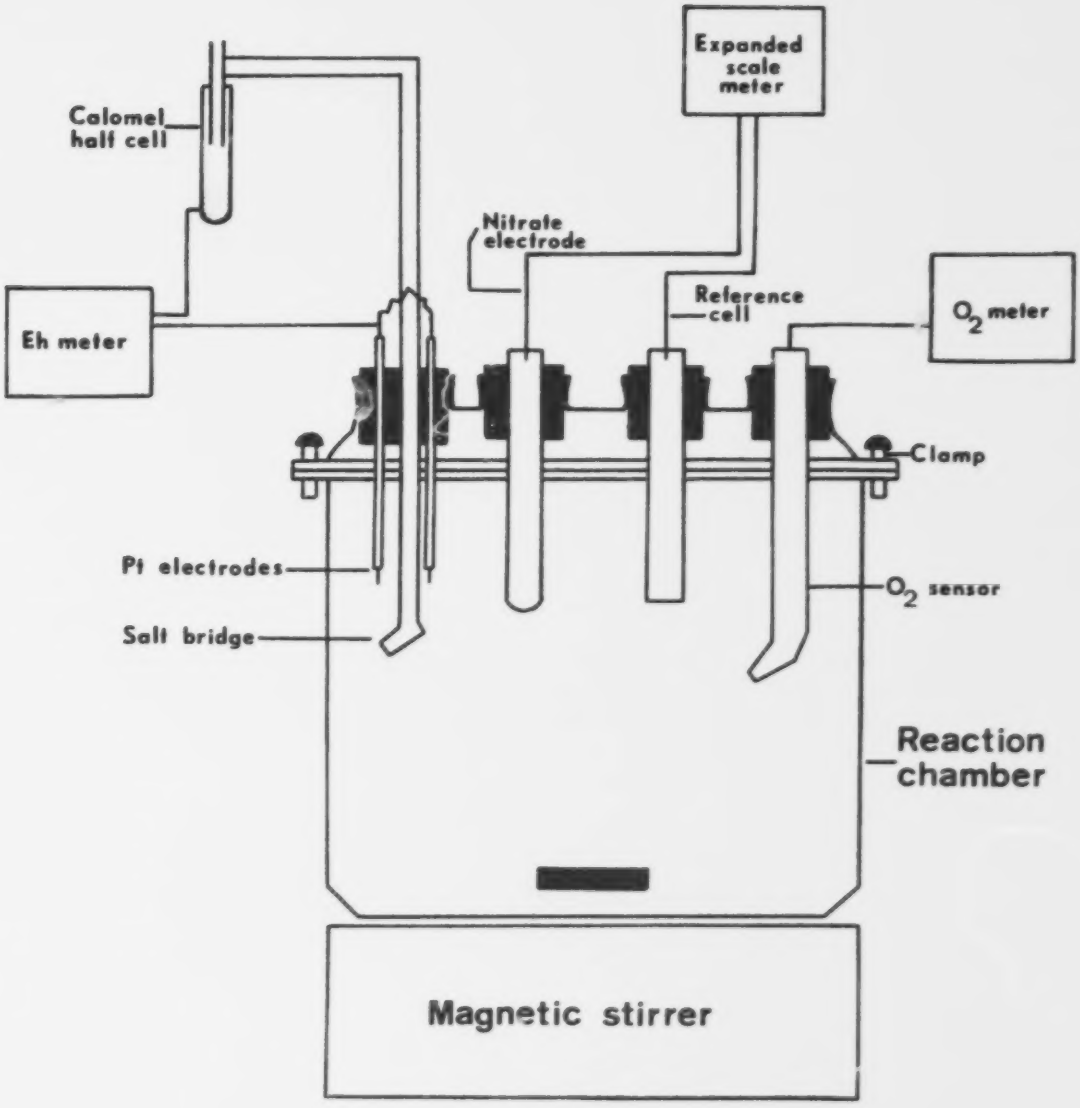


Figure 20. Apparatus used to study nitrate reduction, oxygen consumption and redox potential in stirred suspensions of sediment.

No_3^- Reduction by Anaerobic Soil Suspensions and from Floodwater Dredging Soil

Suspensions (soil:water = 1:2) of each of the four soils were prepared by mixing the soil in a Waring blender with enough NO_3^- as KNO_3 added to the suspension to provide 80 to 100 ppm N (on dry weight basis) to each suspension. For each soil, ten-30g suspensions were placed in test tubes and the tubes purged with Ar. The soils were incubated anaerobically and samples removed periodically for NO_3^- analyses. The soil solution was separated by centrifugation and NO_3^--N measured with an Orion specific ion electrode. Nitrate removal was followed until complete.

Samples of each of the four soils were also placed in one quart wide mouth jars and flooded with approximately 6.5 cm of floodwater that had been sampled with the soil. Nitrate-N was added three times at rates of 50, 90, and 50 ppm to the overlying floodwater and the NO_3^- content of the water routinely determined with an Orion specific ion electrode. When the NO_3^- content decreased to a low concentration the next NO_3^- addition was made and the rate of reduction again observed.

NO_3^- Reduction in Floodwater Separated from Soil

The experimental procedure was similar to the previous experiment except that the water collected from the field sites was placed in the jars exclusive of any soil and NO_3^--N was added at a rate of 100 ppm-N. As a control of floodwater was placed over each corresponding soil and incubated with 100 ppm NO_3^--N added to the water.

Effect of Added O_2 on NO_3 Reduction

The apparatus used for this experiment is shown in Figure 20. Enough

NO_3^- as KNO_3 was mixed into the 1:2 soil:water suspensions to give approximately 40 ppm N and O_2 was bubbled into the suspension until the dissolved O_2 concentration of the water phase reached 16 ppm. Redox potential and concentrations of O_2 and NO_3^--N were monitored as before. In a parallel series the same amount of NO_3^- was added but with no O_2 addition. The small amount of O_2 detected in these suspensions was due to air contamination during mixing. The containers were completely filled with suspension so that no air space existed. As a comparison, an air dry Crowley silt loam (a Louisiana riceland soil) was carried through the identical procedure. The Crowley soil had been stored air dry six months prior to this study.

It should be noted here that in some of the NO_3^- disappearance studies the apparent NO_3^- concentration did not quite reach zero. This is an artifact characteristic of Cl^- interference with the NO_3^- specific ion electrode. Zero NO_3^- concentration is indicated by a low constant NO_3^- reading.

RESULTS AND DISCUSSION

O_2 Removal from Soil Suspensions

Reduction of O_2 for the four suspensions was quite rapid with loss of 16 ppm dissolved O_2 occurring over a range of time from a few minutes to four hours (Figure 21). Soil 1 contained considerable $S^=$ and this highly active reduced material aided in depleting the added O_2 in 15 minutes. The redox potential of this suspension reached a minimum value in about 1 hour. Oxygen addition and subsequent removal had a noticeable effect on the redox potential curves. The point at which O_2

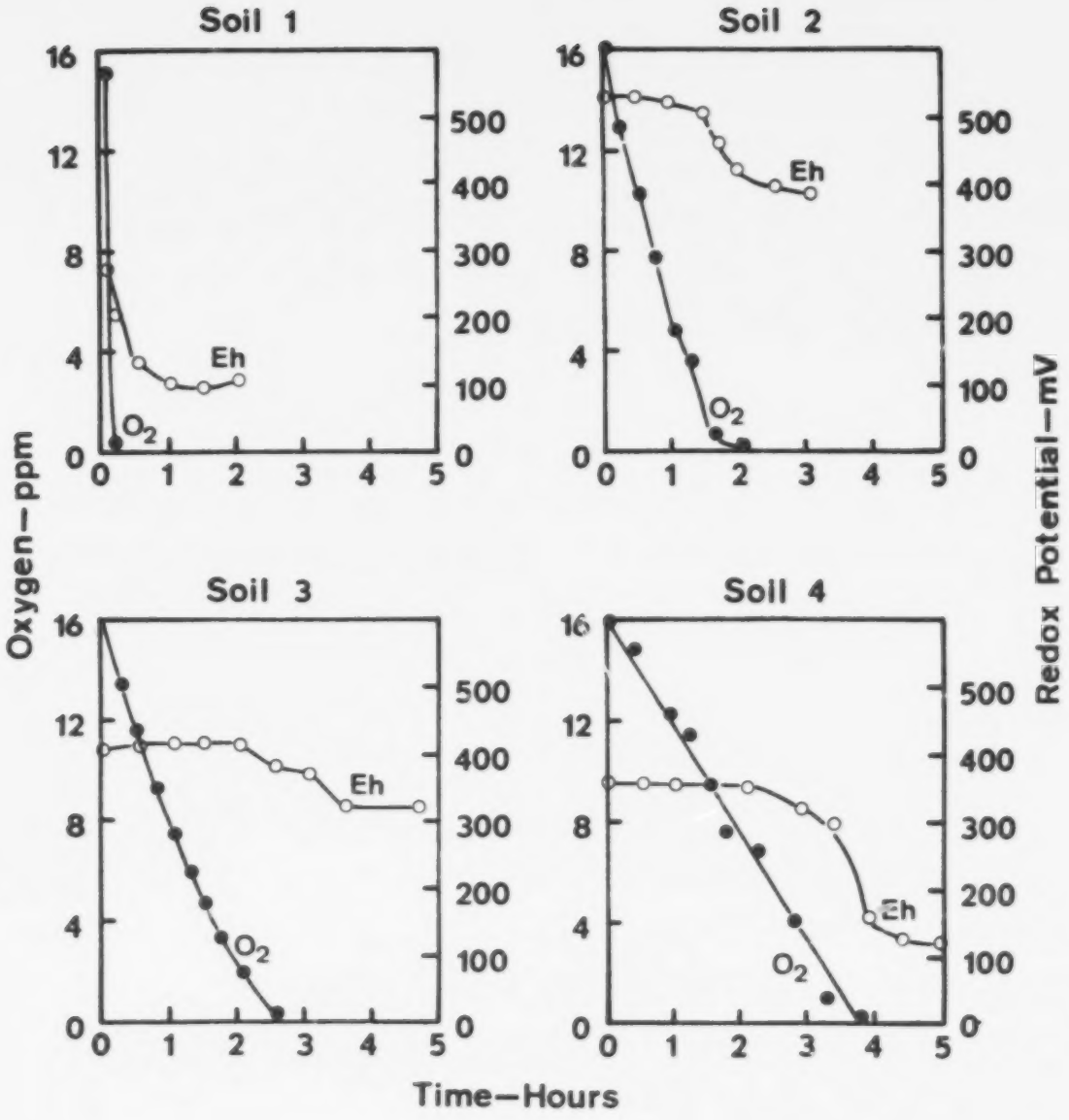


Figure 21. Changes in oxygen content and redox potential with time in stirred suspensions.

disappeared was clearly marked by an inflection in the redox potential curve except for soil 1 which consumed all of the O_2 in a few minutes. The point at which O_2 was depleted was marked by a rapid drop in redox potential after which the potential leveled off probably as a result of being poised by the other soil redox systems. This inflection was particularly apparent in soil 4 which exhibited the slowest O_2 consumption. Since there are a number of oxidation-reduction systems controlling the redox potential in a flooded soil, the disappearance of the oxidized component of one system, such as dissolved oxygen, will result in the potential decreasing rapidly to a new level at which other oxidation-reduction systems, such as those associated with Mn and NO_3^-, will again cause it to be stabilized at the lower potential (Turner and Patrick, 1968). Dissolved O_2 in flooded soil is more difficult to measure in the field than is redox potential and following redox potential changes may be of value in estimating the point at which oxygen is depleted in flooded soils.

NO_3^- Reduction by Anaerobic Soil Suspensions and from Floodwater Overlying Soil

A series of experiments were conducted in which the rates of NO_3^- reduction by the four soils and from the floodwater were measured. These two experiments were designed to determine both the potential rate of NO_3^- reduction and the actual rate that might be encountered under field conditions.

As shown in Figure 22, NO_3^- reduction was rapid in all of the soils with total removal in 1.5 days for soil 1 and 3 and in about 4 days for

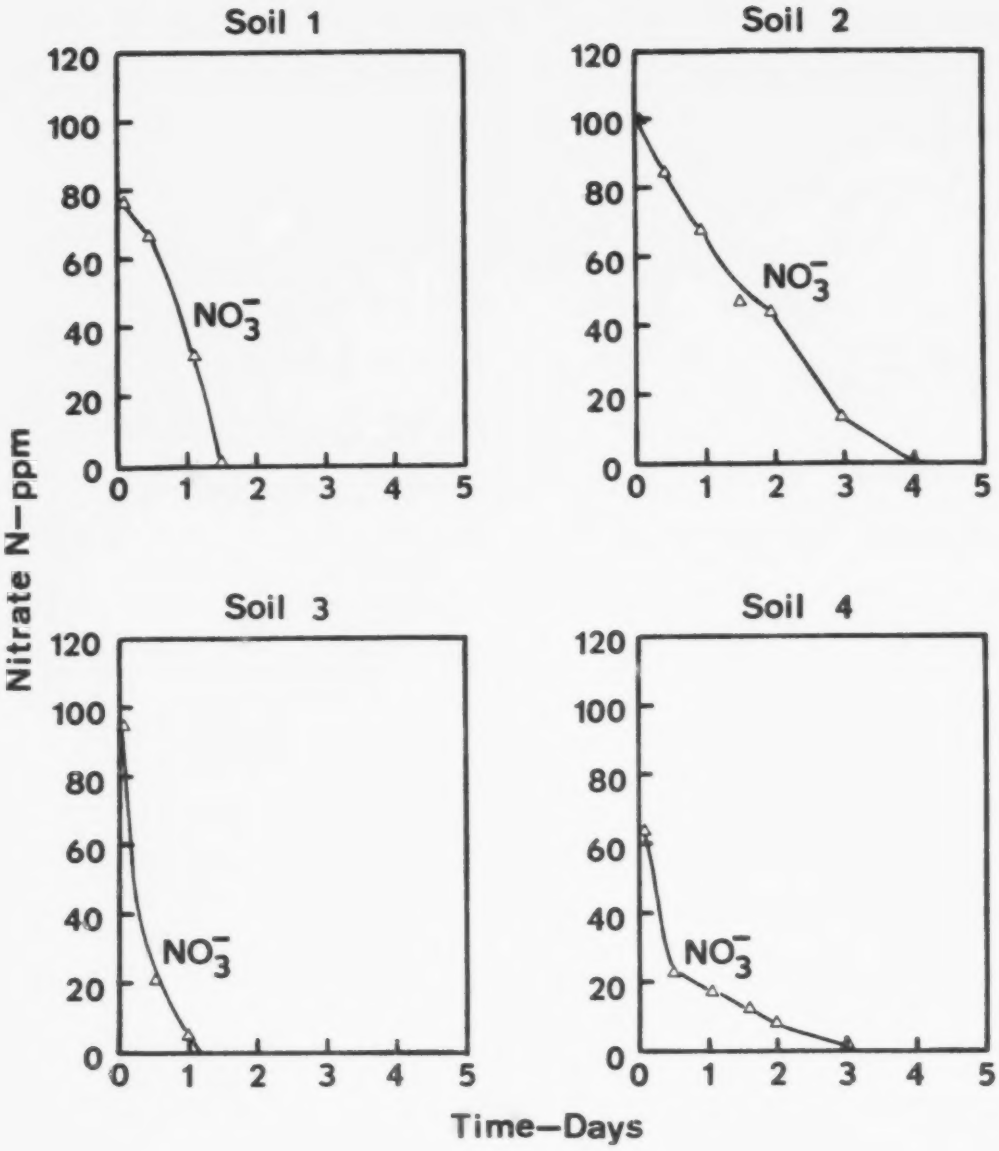


Figure 22. Changes in nitrate content with time in stirred suspensions.

soil 2. Except for soil 4, NO_3^- reduction was essentially linear with time, indicating a zero order reaction. For soil 4 however, NO_3^- reduction was initially rapid but then slowed after one day.

Although the previous experiment showed that NO_3^- mixed with the reduced soil samples was rapidly reduced, no information was provided on removal of NO_3^- from the overlying floodwater. Under field conditions, NO_3^- containing waters are rarely, if ever, well mixed with the soil. Since most NO_3^- in runoff water draining into back swamp areas would accumulate in the floodwater, it is important to determine how rapidly floodwater NO_3^- overlying anaerobic swamp sediments is subject to reduction. Because of the presence of dissolved O_2 in the floodwater, it is possible that NO_3^- reduction in the floodwater would be inhibited by the presence of the more easily reduced O_2. As described in earlier research (Engler and Patrick, 1974), Figure 23 shows that NO_3^- in the floodwater overlying a marsh or swamp soil was lost at a significant rate, although not nearly as rapidly as when mixed with the anaerobic soil.

Rapid NO_3^- removal was noted from the water overlying soil 1 while, as was the case for oxygen removal, soil 2 showed the slowest removal. Probably the most significant finding of this experiment was the ability of the soil-water system to continue to reduce NO_3^- following successive applications of NO_3^- to the floodwater. On a hectare basis, the total reduction of NO_3^- for the entire three-month experimental period amounted to approximately 70 kg/ha. This amount of NO_3^--N is much greater than would be expected to drain into a back swamp from adjacent agricultural areas and indicates that these flooded soils are capable of rapid

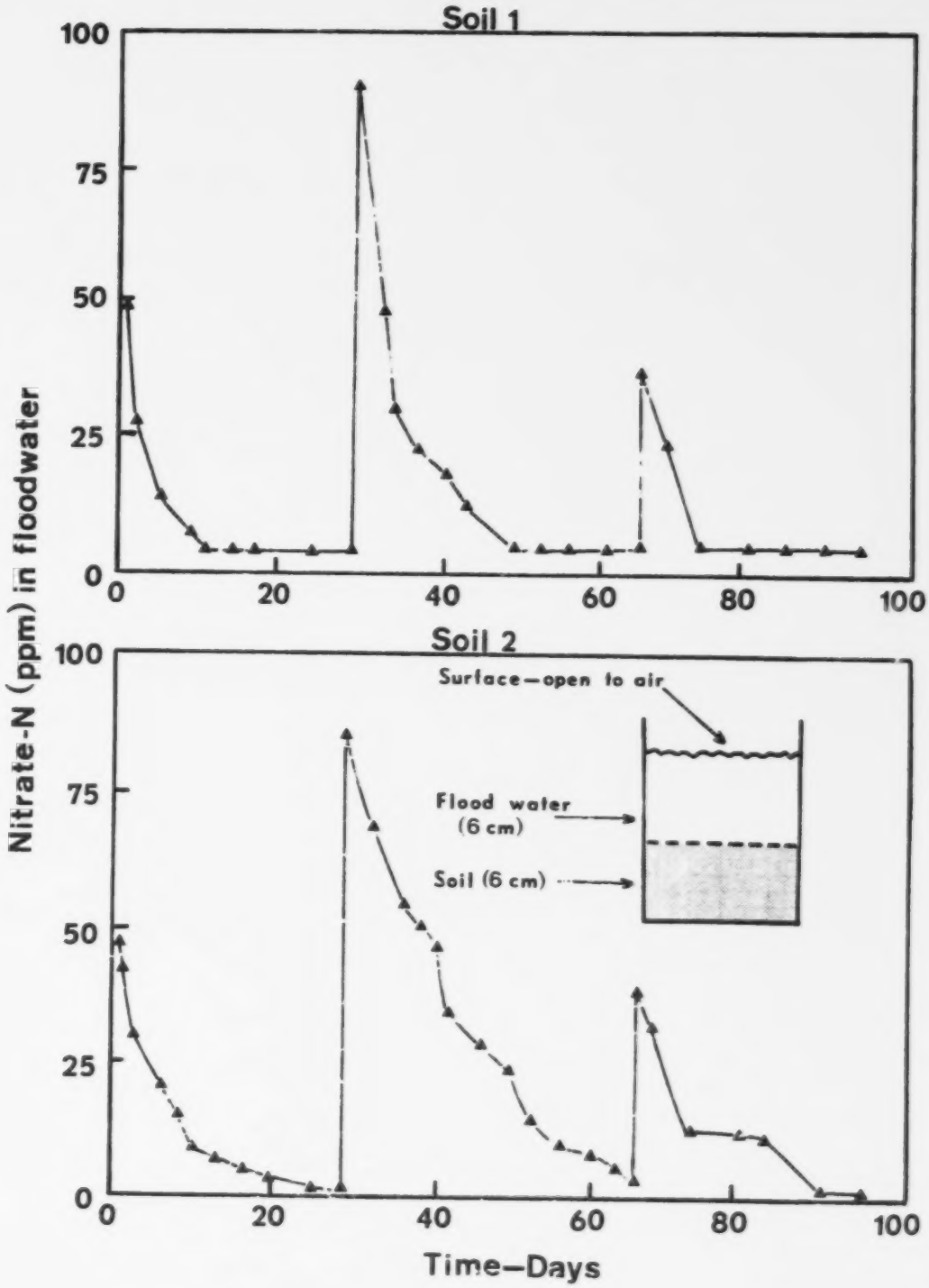


Figure 23. Nitrate removal following repeated additions of nitrate to the floodwater overlying flooded soil.

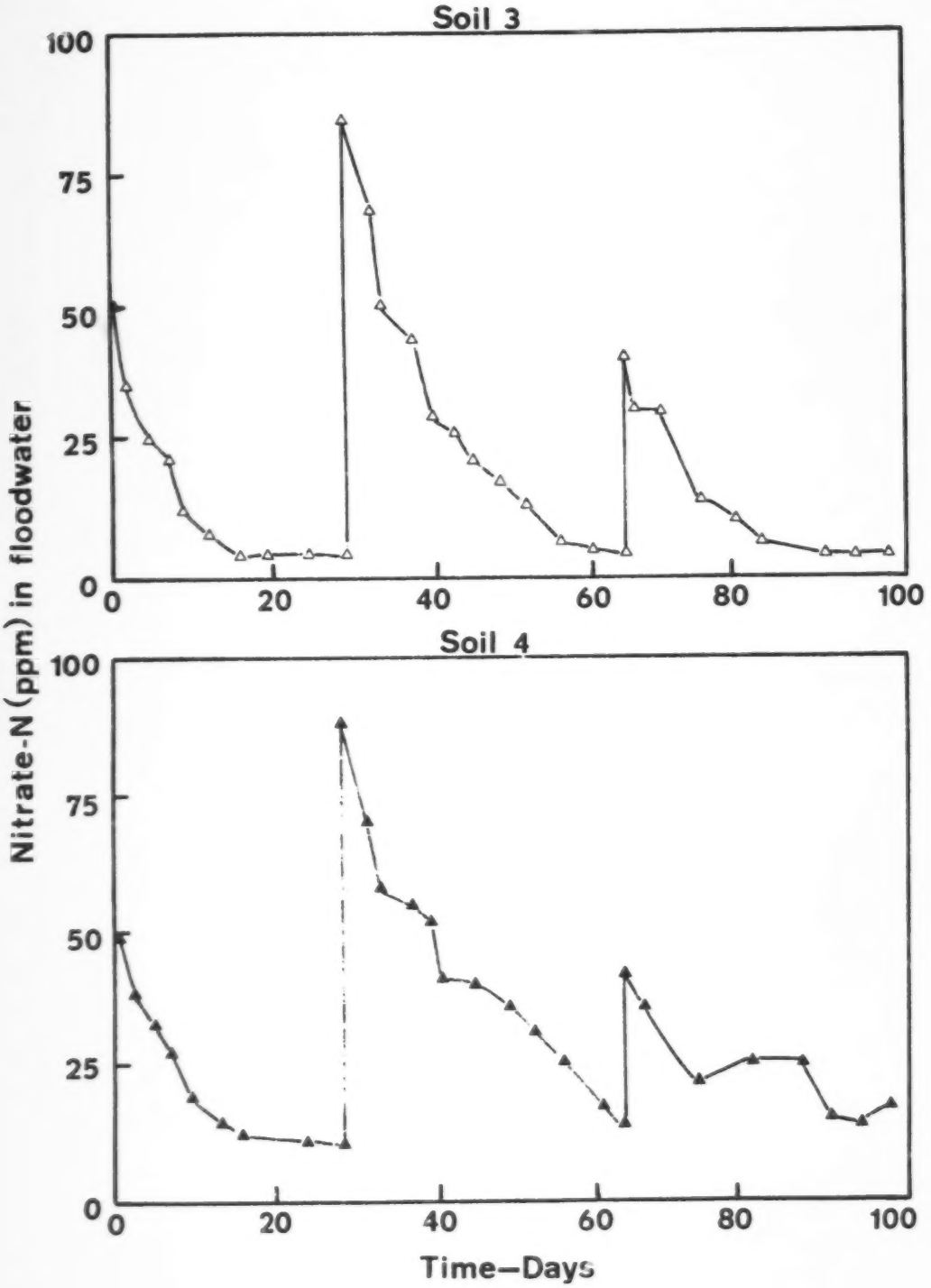


Figure 23 (continued): Nitrate removal following repeated additions of nitrate to the floodwater overlying flooded soil.

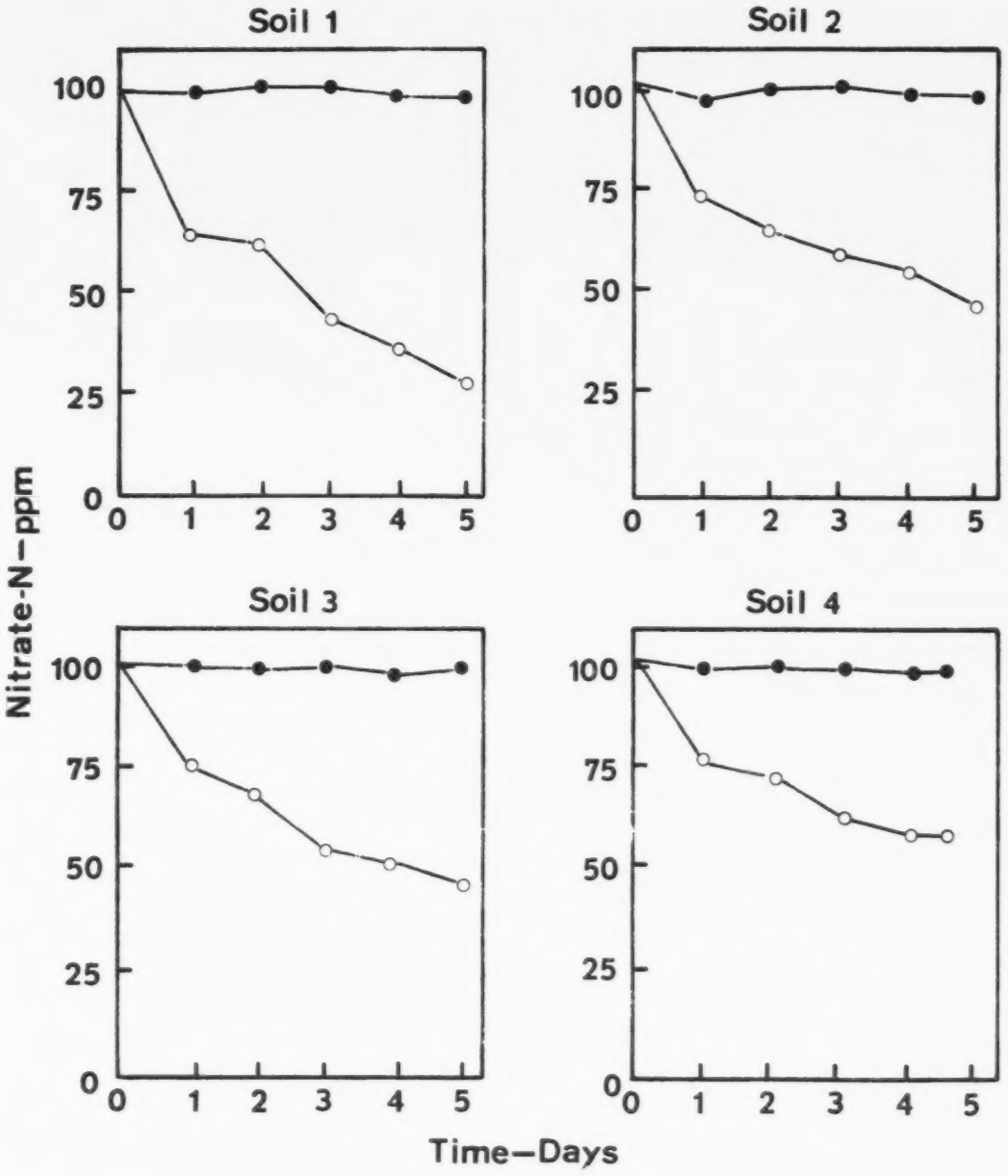


Figure 24. Nitrate removal from floodwater in contact with soil surface and removal from floodwater separated from soil.

removal of NO_3^- draining into the area from adjacent areas.

NO_3^- Reduction in Floodwater Separated from Soil

The results of the previous experiment showed that a rapid depletion of NO_3^- occurred in the floodwater overlying soil. It would be of value to determine if any of this reduction takes place in the floodwater itself or if it is essential for the NO_3^- to diffuse into the reduced soil layer before reduction occurs. An experiment was therefore designed to measure NO_3^- reduction in the floodwater overlying the reduced soil and to also measure NO_3^- reduction in another sample of the same floodwater that had been removed to a separate jar containing no soil. Results of the comparison are shown in Figure 24 with the upper curve of each graph representing NO_3^- content of the floodwater separated from the soil. No NO_3^- disappeared from the separated floodwater. However, NO_3^- disappearance was rapid when the floodwater remained in contact with the soil.

It is of interest to consider the possible mechanisms by which NO_3^- added to a shallow, oxygenated water layer overlying an anaerobic soil is reduced. Little reduction is likely to take place in the oxygenated layer because of the presence of the more easily reduced O_2. Consequently, for NO_3^- reduction to occur, NO_3^- ions must diffuse or leach into the anaerobic soil zone underlying the surface oxidized or aerobic soil layer. There is no significant NO_3^- adsorption onto the mineral soil or organic matter surfaces and the NO_3^- ion is free to respond to the sharp concentration gradient that exists between the floodwater and the reduced soil layer. Though the diffusion of NO_3^- into the reduced layer is

relatively slow, it is continuous since the concentration gradient persists as long as NO_3^- is present in the floodwater. The anaerobic soil layer thus serves as an effective sink for NO_3^-.

The removal of NO_3^- from floodwater does not necessarily mean that all potentially toxic forms of inorganic nitrogen have been removed from the soil-water systems. The NO_2^- ion represents the first product of the denitrification process and an accumulation of NO_2^- could be more toxic than the equivalent concentration of NO_3^-. All reported studies have shown, however, that under conditions comparable to those in the field, only trace amounts of NO_2^- (2 to 5 ppm) accumulate in flooded soils. Apparently, the reduction of NO_2^- proceeds at a potentially higher rate than does the reduction of NO_3^-, since NO_2^- reduction is seldom a rate limiting step in denitrification.

Effect of Added O_2 on NO_3^- Reduction

In general, 16 ppm added O_2 did not appear to significantly hinder NO_3^- reduction in the soil suspensions. As shown in Figure 25, NO_3^- reduction began immediately after NO_3^- addition in both oxygenated and nonoxygenated samples and most of the added NO_3^- had disappeared by the end of 24 hours. The added O_2 was depleted in 0.5 to 5 hours after addition with the $S^=$-containing soil (soil 1) consuming O_2 most rapidly and soil 4 requiring the longest time for O_2 removal for the swamp soils. The agricultural mineral soil required 6 hours to consume all the O_2. The redox potential of the suspension again responded markedly to the disappearance of oxygen. At the time of O_2 depletion, the redox potential decreased sharply. This inflection in redox potential was discussed

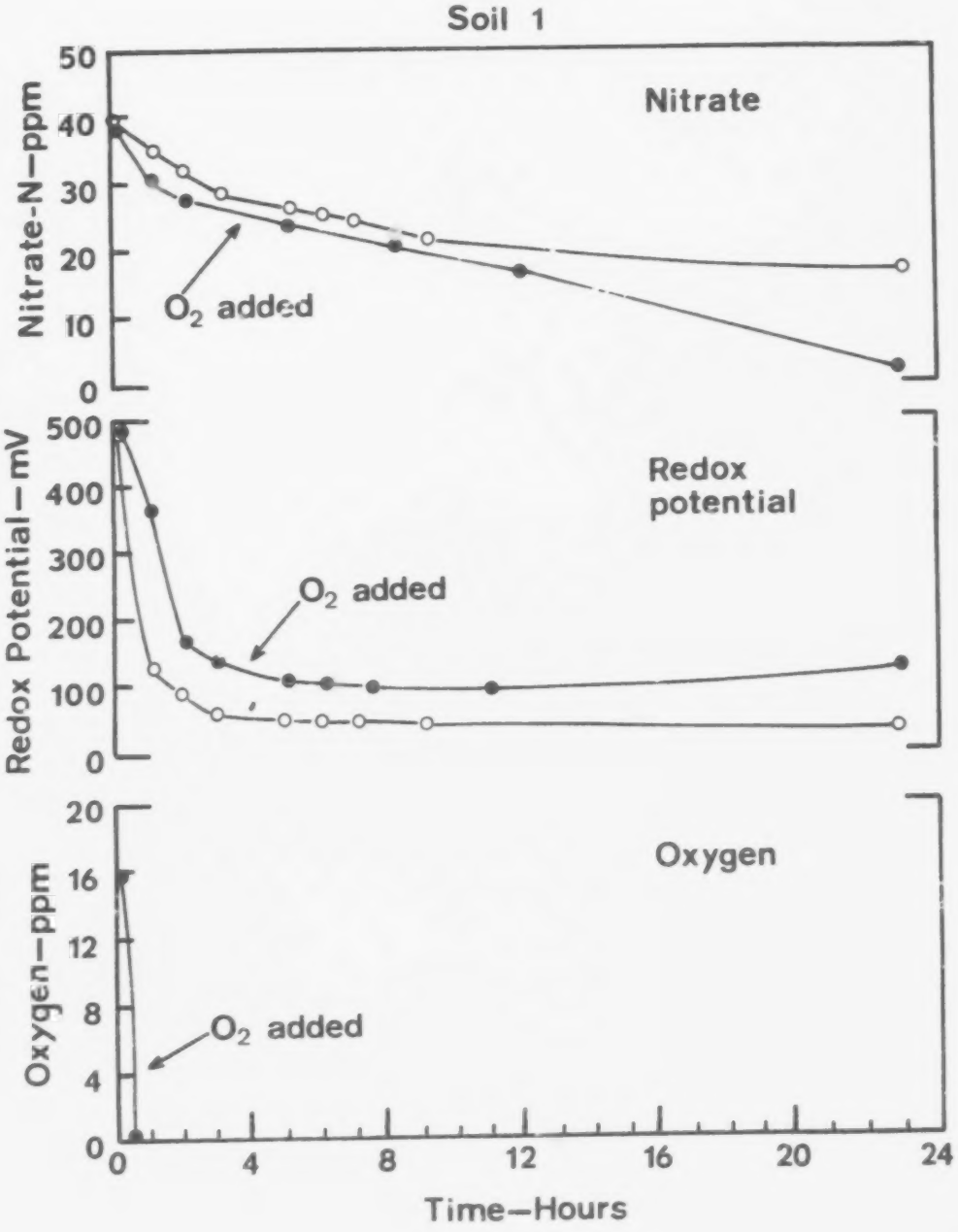


Figure 25. Effect of added oxygen on nitrate reduction and redox potential in stirred suspensions.

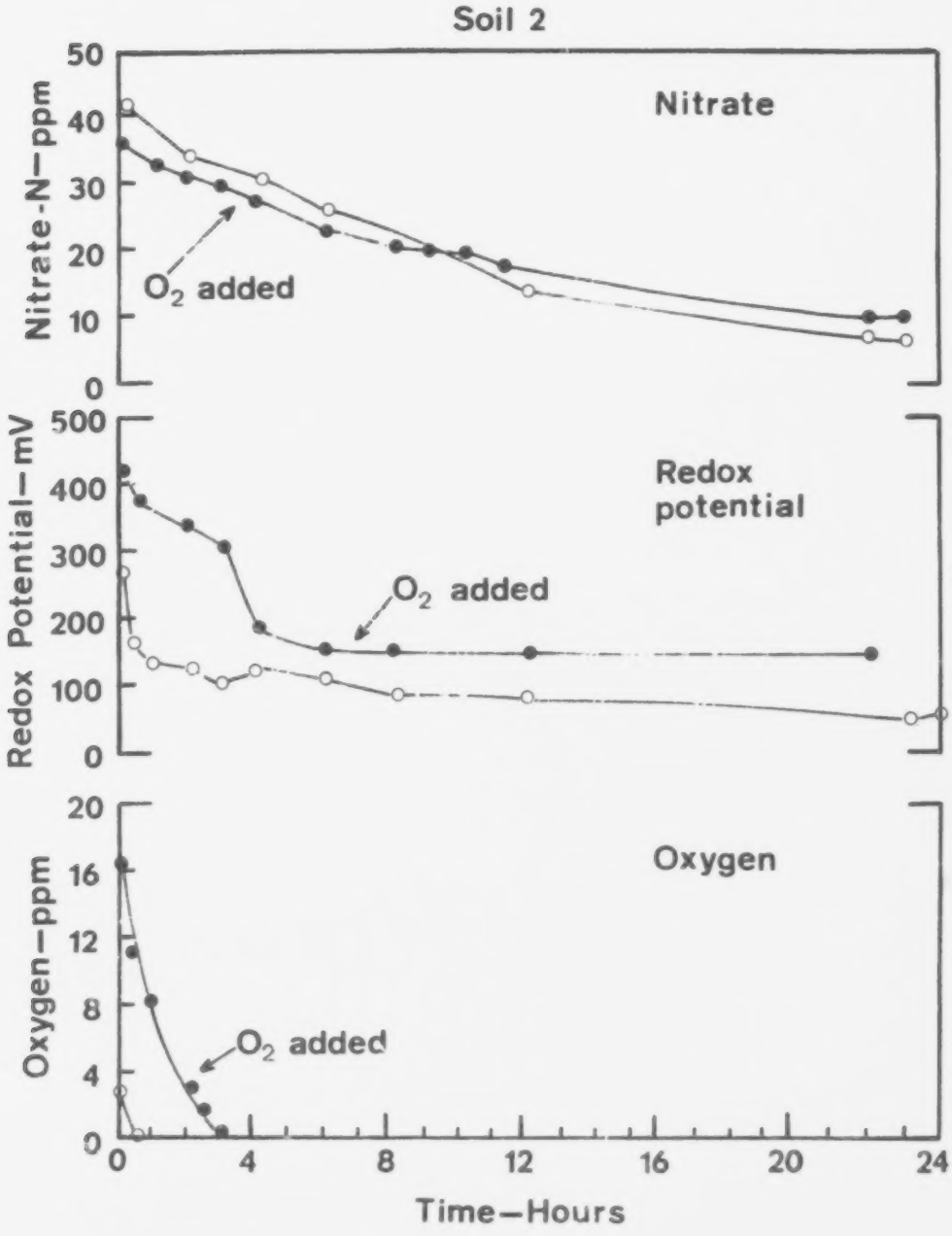


Figure 25 (continued): Effect of added oxygen on nitrate reduction and redox potential in stirred suspensions.

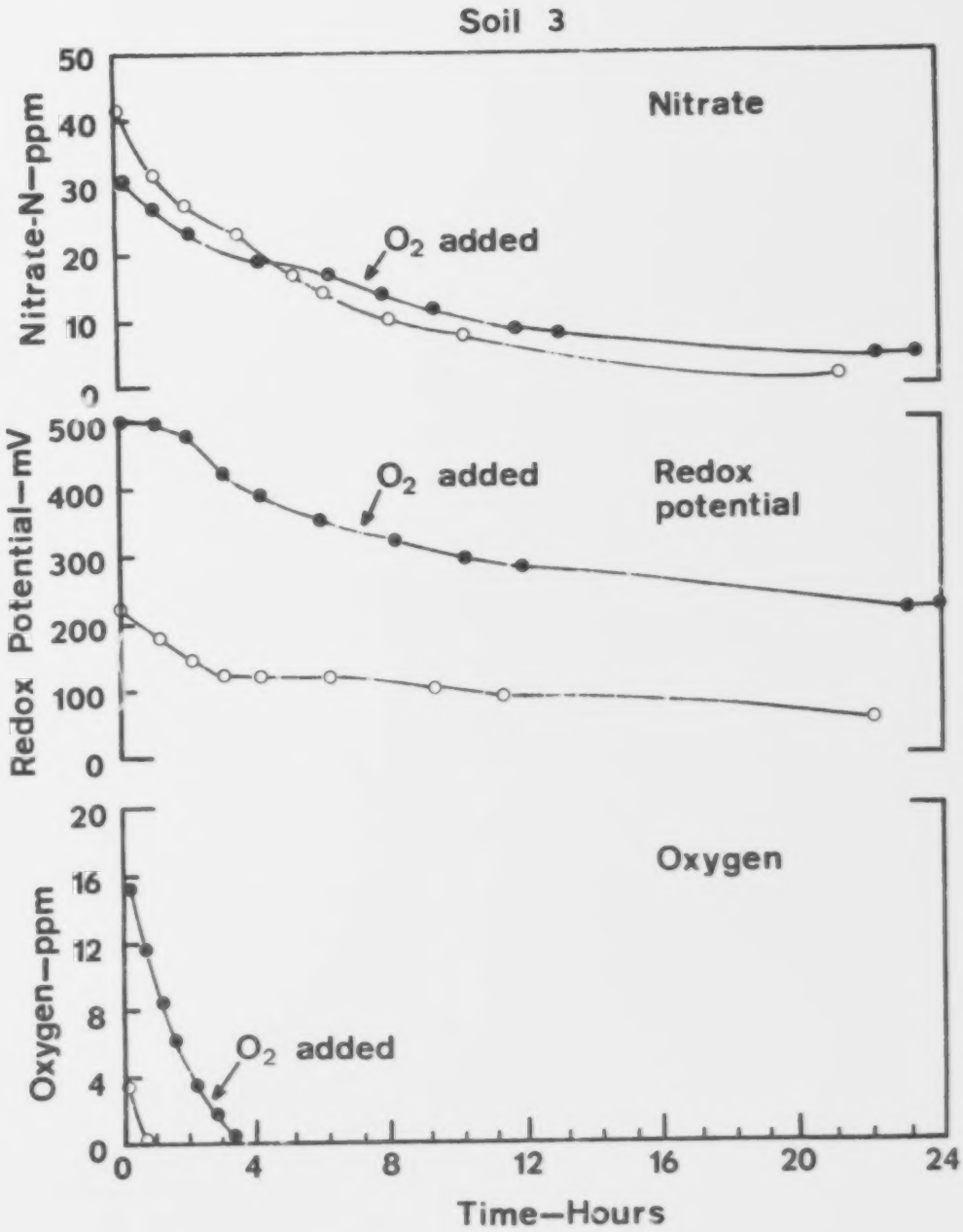


Figure 25 (continued): Effect of added oxygen on nitrate reduction and redox potential in stirred suspensions.

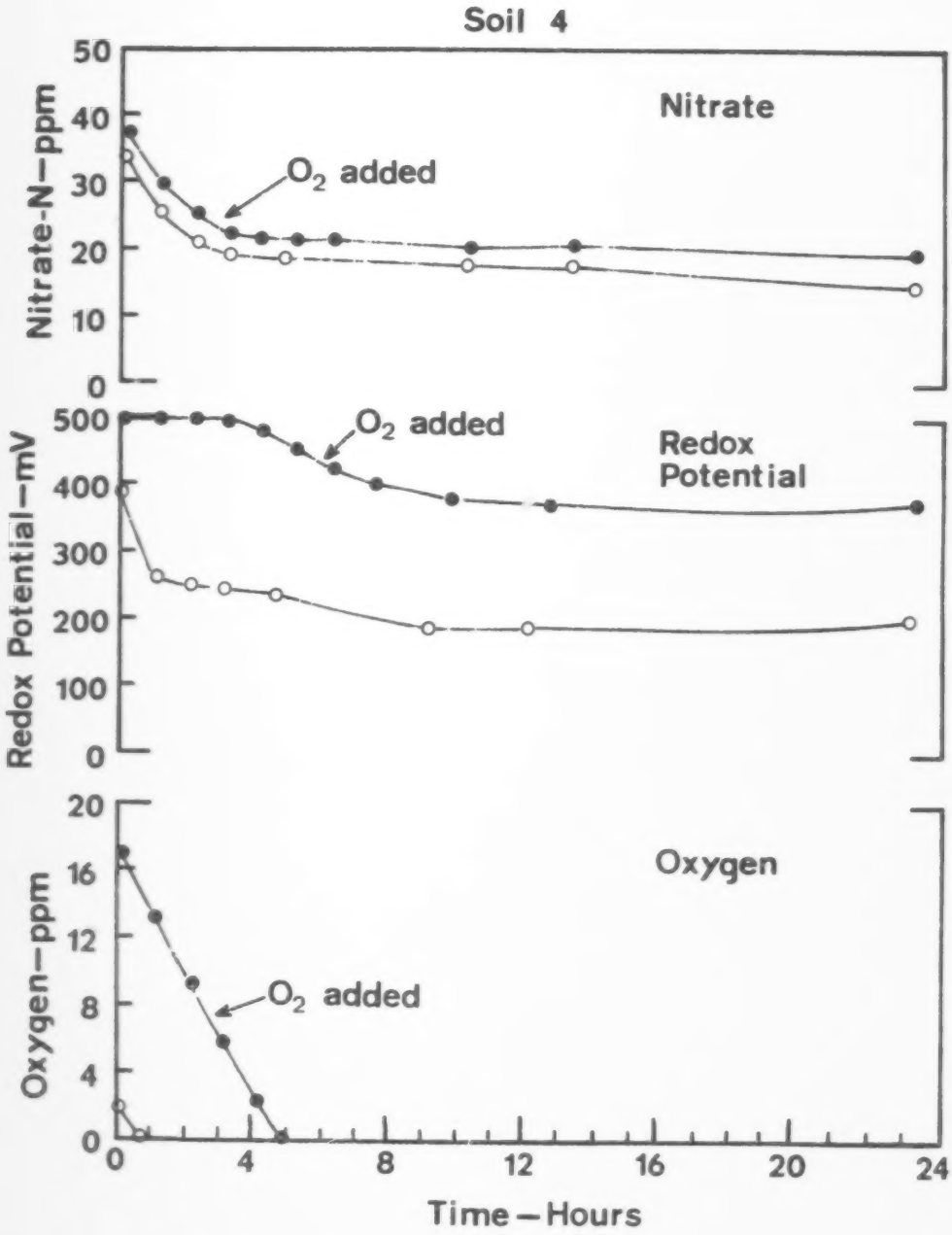


Figure 25 (continued): Effect of added oxygen on nitrate reduction and redox potential in stirred suspensions.

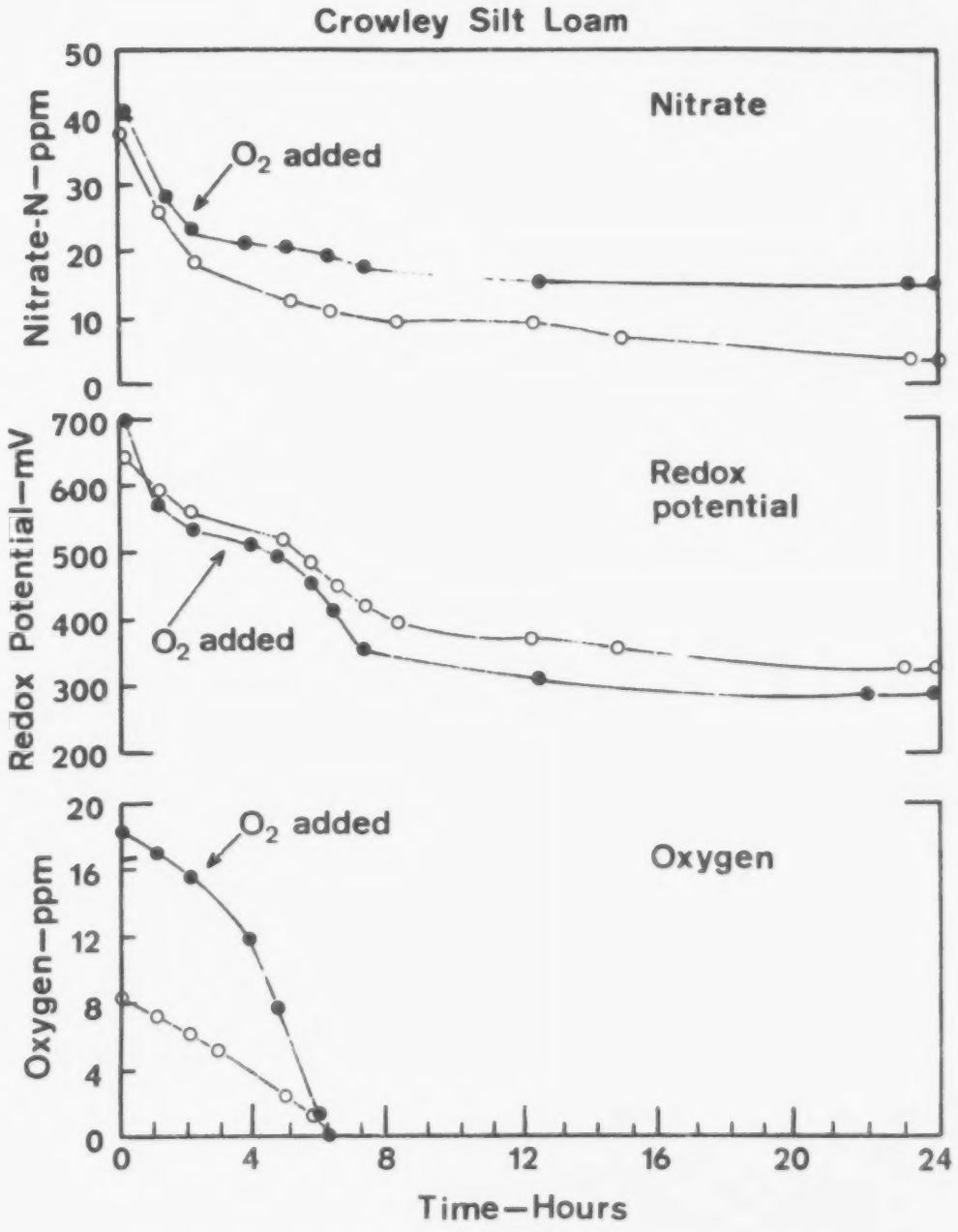


Figure 25 (continued): Effect of added oxygen on nitrate reduction and redox potential in stirred suspensions.

in a previous section, however, no change in NO_3^- removal rate could be detected at this point. The Crowley soil which was thoroughly oxidized at the beginning of incubation showed a pattern similar to that of the anaerobic soils.

Soil 1 which had previously shown the most rapid reduction of O_2 and NO_3^-, also showed the same trend in this experiment. Demand for O_2 was so rapid that an inflection in the redox potential curve could not be detected. There was, however, about a 75 mV higher potential noted in the oxygenated series. Soils 2, 3, and 4 also showed no distinct effect of added O_2 on NO_3^- removal from the suspension, however the redox potential curves showed the characteristic inflection points as O_2 disappeared. Soils 3 and 4 exhibited highly poised redox potentials (about 200 mV higher where O_2 was added) while soil 2 showed a difference in Eh of about 75 mV between the O_2 treated and untreated systems. The addition of 16 ppm of O_2 (twice that of average atmospheric O_2 saturated suspensions) did not appear to inhibit NO_3^- disappearance in any of the soil suspensions, but did result in a characteristic inflection in the redox potential curves. It should also be mentioned that this was a very small amount of O_2 in terms of the reducing capacity of these anaerobic soils.

The agricultural or comparison soil (Crowley soil) had a much higher initial redox potential which was characteristic of a very oxidized soil system and contained significant atmospheric O_2 at the beginning of incubation as shown in the curve where no O_2 was added. The same characteristic inflection of redox potential at the time of O_2 depletion was again demonstrated. As in the swamp soils, NO_3^- reduction was not significantly affected by O_2 addition.

CONCLUSIONS

The experiments carried out in this study demonstrate that the flooded swamp and marsh soils in the interior swamp and coastal marsh areas of Louisiana have a high capacity for reduction of NO_3^-. Nitrate added either to the shallow floodwater or mixed in the soil rapidly disappeared under laboratory conditions. Nitrate reduction does not take place in the floodwater, but is dependent on the NO_3^- moving downward into the anaerobic soil layer. The NO_3^- reduction capacity is much greater than is required to handle any amount of NO_3^--N that is likely to be present in these areas, either from oxidation of ammonium that has been released from organic matter or from NO_3^- derived from local runoff from adjacent agricultural areas. Nitrate reduction does not appear to be inhibited by the presence of a small amount of molecular O_2 in flooded soils.

SECTION VII

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